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STUDIES ON STAPHYLOCOCCUS AUREUS IN HELSINKI

THE DISTRIBUTION OF PHAGE TYPES AND ANTIBIOGRAMS, AND THEIR ASSOCIATION WITH CERTAIN OTHER CHARACTERISTICS

BY

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PREFACE

First and foremost, I wish to express my deepest gratitude to my teacher in Serology and Bacteriology, Professor K. O. Renkonen, M.D., for introducing me into the fascinating world of experimental research. His genuine enthusiasm about new discoveries in various branches of science has been a source of inspiration for his numerous pupils.

When the present work was at its initial stage, I was fortunate enough to get acquainted with the problems and available techniques of staphylococcus research in the Gade Institute, University of Bergen, Norway, under Professors Th. M. Vogelsang, M.D., and Per Oeding, M.D., for whose generous help I wish to acknowledge my warmest thanks.

This work has been carried out during the years 1958 and 1959 in the Municipal Bacteriological Laboratory, Helsinki, Head, Dr Odd Wager, M.D., and, for a smaller part, in the Department of Serology and Bacteriology, University of Helsinki, Head, Dr K. O. Renkonen. I want to thank both of them for placing the facilities of their laboratories at my free disposal. I want to thank specifically Dr Wager for his advice, suggestions and unfailing interest in the subject during innumerable discussions.

The subject of the investigation is a natural consequence of the close co-operation between the Helsinki Municipal Bacteriological Laboratory and the Aurora Hospital. The necessity for staphylococcus research had long been felt and expressed on many occasions by physicians in the different departments of the hospital; among them I want to extend my especial gratitude to Dr E. Klemola, M.D.

For the material I am indebted to the heads, to several doctors, and to the nursing staff of many Helsinki hospitals, of the

Student Health Service, and the microbiological laboratories at Turku and Lappeenranta.

Finally I wish to acknowledge my gratitude to my co-workers, particularly to Miss Birgit Östervold in the Gade Institute, to Mrs Kirsti Jäderholm, Dr O. Mäkelä, M.D., Dr R. Mäkitalo, M.D., Miss Eeva Raatikainen, and Dr O.-V. Renkonen in the Department of Serology and Bacteriology, to my technical assistants Miss Kaija Saarikivi, Miss Maija-Liisa Saarikivi, and Mrs Lilja Ziegler, and to the whole staff in the Municipal Bacteriological Laboratory, with whom it was a pleasure to work.

This study would not have been possible without the material help received from the Yrjö Jahnsson and Eemil Aaltonen Foundations.

October 1959

P. M.

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INTRODUCTION

While the severity and consequently the importance of most infectious diseases have been drastically reduced during the last two decades, staphylococcus infections have emerged as increasingly prominent. And whereas the mortality rate as well as the morbidity of several infectious diseases has been brought down near to zero by chemotherapeutic treatment on the one hand and by the prevalence of vaccinations on the other, the staphylococcus has retained its ubiquitousness and disease-inciting power undiminished. The question argued about at present is whether the number of infections caused by *Staphylococcus aureus* is greater than two or three decades ago and whether its virulence has increased, even though the question may well be unanswerable. It is beyond dispute, however, that staphylococcal infections continue to be a frightful, major problem.

Neither advances in, nor the generalized use of, chemotherapeutics, nor the increasingly higher standard of hospital care have succeeded in eliminating the staphylococcus problem. On the contrary, the staphylococcus has turned out to be extraordinarily adaptable and consequently capable of adjusting itself to rapidly changing conditions. It has developed strains that are resistant to the drugs in use, and shows a predilection for the modern hospital as its breeding place.

The streptococcus, which some twenty years ago was the chief cause of wound infections, has now been deprived of its first place by the staphylococcus. A similar shift is taking place in the case of puerperal infections, and neonatal infections are for the most part of staphylococcal origin. An increasing number of cases of pneumonia, otitis, meningitis and sepsis are now being caused by staphylococci instead of by the previously

common etiological factors such as pneumococci, meningococci etc.

A considerable proportion of staphylococcal infections is due to bacteria that are resistant to antibiotics. At the present time there are hosts of antibiotic-resistant staphylococci in many hospitals in the first place. On the other hand, patients at these hospitals are often treated by antibiotics. By the agency of these two factors antibiotic-resistant strains tend to be singled out into the injuries of hospitalized patients especially, with the result that lesions which have become infected in hospitals are more difficult to treat than those which have become infected elsewhere.

No wonder, then, that the staphylococcus problem, particularly the problem of those staphylococcal infections which are acquired in hospitals has of late years attracted attention the world over. On the subject a vast literature has accumulated in the journals both of the various branches of clinical medicine and of bacteriology and epidemiology as well; the theme has also been discussed at large symposiums (1—6). The final problems, however, both of the prevention of hospital-acquired staphylococcal infections and of the varying virulence of staphylococci still remain largely unsolved; and the conferences all wind up by declaring that, while it is imperative that every practical measure should be taken in order to prevent staphylococcal infections in hospitals, continued research into staphylococci is of the utmost importance.

REVIEW OF LITERATURE

THE OCCURRENCE AND CLINICAL SIGNIFICANCE OF *STAPHYLOCOCCUS AUREUS*

Staphylococcus aureus seems to occur in a close association with the human population, being more prevalent in densely populated areas and communities than elsewhere (124). Its chief habitat — from the microbe's point of view — is probably the upper respiratory tract of man. Rather widely different estimates of the frequency of human nasal carriers of *Staphylococcus aureus* are quoted, most of the differencies being due to differencies in isolation techniques. The true nasal carrier rate is perhaps somewhere about 75 per cent (165).

Except in the upper respiratory tract, the staphylococcus* is found as a saprophytic organism on the human skin and in the feces. The skin carriage probably means the result of contamination from the nose (165). Usually, 5 to 24 per cent of skin swabs have been found positive for *Staphylococcus aureus.* Fecal carriers seem to be more frequent among infants (100 per cent under 6 months of age, 50 per cent between 6 and 12 months) than among adults (20 per cent) (35). Such high rates, however, have probably been attained with a very efficient isolation technique.

In the usual infections caused by the staphylococcus, *i.e.* purulent infections of various kinds, the microbe is quite obviously an etiological factor. In some dermatological conditions, however, such as infected eczema, its role is often secondary. Nevertheless, isolation of staphylococci from pus samples of this sort is significant with regard to the treatment of the patient.

* In this study, »staphylococcus» will often be used to indicate the species *Staphylococcus aureus* only.

In specimens that generally contain staphylococci along with other bacteria the significance of it is not easy to estimate. A principle which may be taken as a rough guide is that the staphylococcus is to be considered of etiological importance only when it definitely outnumbers the remaining flora of the sample. This holds good *i.e.* of expectoration and feces. In pharyngeal specimens *fewer* staphylococci have been found in children suffering from respiratory infections than in healthy ones of the same age (77, 154). In infants, the staphylococcus is sometimes, though perhaps not very often, the cause of a respiratory infection; in these cases, too, very uncertain conclusions can be drawn from pharyngeal specimens (77, 135). The majority of staphylococcal pneumonias in children occur in the youngest age groups, under 4—6 months (76).

TYPING OF *STAPHYLOCOCCUS AUREUS*

DIFFERENT METHODS OF TYPING

For a long time researches into the subject of *Staphylococcus aureus* have been hampered by the very ubiquity of the species. It has been impossible to trace the sources and vectors of infection when more than a half of those around the patient, for example, are carriers of the potentially pathogenic *Staphylococcus aureus.*

So it has become very necessary to develop methods by means of which this species can be divided into hereditary types; indeed, attempts at devising practicable methods for this purpose have been succesful. The methods are of four chief types:

a) Those which are based on biochemical methods, such as
serum opacity test (168, 136), and
egg yolk opacity test (56).

b) The method which is based on the sensitivity of different staphylococci to various chemotherapeutic agents; the typing results are given as »antibiograms» for each strain (98, 106).

c) Serological typing, only practicable since the introduction by Cowan of the slide agglutination technique (43). In its present form it is based on reactions given by 8 absorbed anti-

sera as worked out by Oeding (97). Besides him, especially Grün (60) and Brodie (32) have used this method.

d) Typing by means of bacteriophages. The method in general use at present has evolved out of Fisk's technique for isolating staphylococcal bacteriophages (51) and from the pioneering work of Wilson and Atkinson (164).

To date the above methods have been discussed both from a practical standpoint and comparing the advantages and drawbacks as well as the interrelations of the several methods in many surveys (106, 98, 99).

The following are some of the requirements a good method of typing is expected to meet.

1. First and foremost, the »types» determined by the method must be distinct and constant. That all strains ought to be typable follows by implication.*

2. In the case of staphylococci, in particular, the method must be capable of differentiating between a large number of types.

3. Technically, it must be reasonably easy to examine strains in large numbers.

4. The reagents must keep well and their identity must be repeatable from batch to batch.

The biochemical methods do not fulfil requirement No. 2, being each capable of distinguishing 2 types only. For this reason there is not much use for them in epidemiological work.

The antibiogram, which for obvious reasons is the favourite method of the clinician, meets requirements No. 3 and No. 4, but Nos. 1 and 2 poorly. It cannot be used to differentiate very many types, but it is readily selective of »hospital strains» from »wild type» staphylococci. It is the strains showing multiple resistance that can be regarded as potentially dangerous in the event of a hospital epidemic (157). The sensitivity of a given strain to antibiotics is not a constant characteristic; there is always the

* The term »non-typable», besides its proper application to strains that cannot be examined at all by a given method — such as those which show spontaneous agglutination in serological typing based on the agglutination in different sera —, is also used of strains that give a negative reaction in regard to the reagents used for typing (serum or phage for example).

possibility of more resistant mutants gaining ascendancy during treatment.

All the above requirements are satisfied by *serological typing,* to which, however, *typing with phages* is in many respects superior. True, serological types are probably very stable, when strains of the same origin are studied, whether several strains from the same person, isolated at different sites and times, or strains stemming from the same epidemic, or different subcultures derived from the same source in the laboratory, but so are phage patterns, too (see, however, p. 17). Both methods presuppose that the limits are known between which variations are non-significant in type differentiation. In this respect considerably more empirical information has accrued about phage typing. The phage typing method is more suitable for large-scale mass examinations because a bigger share of the operations can be trusted to relatively unexperienced persons. Preparation of the reagents for the serologic typing is perhaps less laborious, but also less is known about the ease of obtaining similar batches than in phage typing that has been carried out a long time under careful control.

THE PHAGE TYPING

— *The Method.* In consequence typing with bacteriophages is the method of choice in epidemiologic staphylococcus research at present. The Subcommittee on Bacteriophage Typing of Staphylococci has been set up under the authority of The International Bacteriological Nomenclature Committee. This body is charged with exchanging pertinent information and appointing a central laboratory to supply the national typing laboratory of each country with strains of bacteriophages and staphylococci. The functions of such an International Reference Center have hitherto been performed by The Staphylococcus Reference Laboratory; Head, Dr R. E. O. Williams; address, Colindale Avenue, London, N.W. 9. (10). The subcommittee also arranges tests to compare phage typing results obtained in the different laboratories. In this way the typing results of the different laboratories can be compared and an eye kept on the occurrence of given phage types in various parts of the world. Stringent

control of every phase of work is absolutely necessary, which is shown by the fact that despite all the efforts a batch unidentical with the prototypic phages has slipped away from a major laboratory. Thus the widely known type 80/81 has appeared as 52/42B/44A/81 in many reports published in the U.S.A. and Canada (27).

The details of the technique of phage typing are largely those described in the work of Williams and Rippon, 1952 (161). A few slight modifications have been adopted since, some phages, for instance, have been found to require an excess of calcium ions (111), and it is advised to add calcium to the culture medium. Laboratory notes describing the current technique in detail are obtainable from The International Reference Center (11). The same basic set of 21 phages is recommended for universal use. Of course, additional phages, either newly isolated ones or such as have proved their suitability under local circumstances, may be used by the laboratories.

— *The Overall Sensitivity to Phages of Staphylococci.* Only the coagulase positive *Staphylococcus aureus* is sensitive to the typing phages. The phage is usually used at the so-called routine test dilution (R.T.D.), which is chosen so as to produce a strong lysis with the homologous staphylococcus. If, however, by this means a lysis is not achieved with any of the test phages, a repeated test is made using 1000 × more concentrated phages; the greater part of strains is now lysed by some phage(s). There is no clear idea of the significance for strain differentiation, if any, of the degree of sensitivity to phage. It is commonly believed that differentiation cannot be established on this basis (156). Some workers use the phage at 100 × R.T.D. instead of at 1000 × R.T.D. or in addition to it thus achieving greater group specificity because of fewer reactions (3, 104).

— *Lytic Groups of Staphylococci.* A given staphylococcus strain may be lysed by one or several phages. In the latter case certain predilections are found to obtain among the phages. This fact has been turned to account by dividing phages into four groups; those phages which oftenest exhibit associated lytic reactions are considered to belong to the same group. The groups are I, II, III, IV and they correspond roughly to Cowan's

serologic groups (63). *N.B.* At the early stages of typing different groupings were used, *e.g.* 148, 28. Moreover, there are phages, called miscellaneous, that do not belong to any of these four groups.

A staphylococcus is said to belong to the same group as that phage by which it is lysed. There are, however, strains of staphylococci that are lysed by phages of two or more different groups, and whose group accordingly remains indeterminate. Fewer of these mixed-group strains are encountered at the routine test dilution of phages than when a 1000 × higher concentration is used. Pöhn (104), who has discussed this point, distinguishes primarily atypical staphylococcus strains and atypical phages on the other hand, *i.e.* such as do not absolutely belong to any one group.

— *Inhibition Reactions.* In higher concentrations (1000 × R.T.D.), some staphylococcus strains and some phages give, not true lysis but an inhibition of growth of staphylococci instead. Such an inhibition may also occur in conjunction with a lower degree of lysis. The mechanism of the inhibition is not fully understood (161, 115), but it appears to be as specific as the lysis proper and may thus be used as an adjunct for the purpose of strain differentiation (11, 115).

— *Reporting of Results of Phage Typing.* As the phage pattern, or 'type', of the staphylococcus those phages are mentioned in succession which have brought about a strong lysis with it. This practice has the definite disadvantage that any additional weaker reactions are disregarded (their occurrence is indicated with the + sign, though). The truth is that the reactions are not so constant as to preclude variations in strength of reaction of a strain with one or more phages. Under such circumstances as these a change from a reportable to not reportable reaction readily occurs. As a consequence one and the same strain will be reported by means of two patterns so dissimilar that a person who is not able to make reference to the laboratory notes showing the positions of any weak reactions cannot possibly know that the two patterns stand for the same strain. In order to avoid this drawback it has been suggested that all reacting phages in sequence of strength of reaction

should be reported (10)*; the subcommittee however, has not considered amendments practicable in spite of the obvious disadvantages of the present system of reporting (10).

— *The Value of Phage Patterns in Strain Differentiation.* In this matter there are the recommendations of Williams to go by, and they are based on very extensive materials (161, 156, 11). The leading principle is that two staphylococcus strains can be regarded as different if they differ by two major reactions. Minor differences—variations from strong to weak lysis or from weak to none or *vice versa*—are not important. This is a stringent requirement, however, and there are certainly a greater number of distinct types in existence than can be detected in this way. In an actual epidemiological investigation, therefore, which involves testing a large number of staphylococci of identical origin and affords knowledge of the range of variation of »the epidemic type», a more efficient classification of strains as identical or distinct is feasible (99).

— *Lysogenicity.* In phage typing there is an important theoretical source of error: most staphylococcus strains carry some prophage or prophages rendering the carrying strain resistant to the phage(s) in question. This resistance is not a constant property, however, it being possible for the staphylococcus to lose its prophage, to acquire another or several others, or to have it replaced by another. Furthermore, a single prophage may make the strain simultaneously resistant to a number of phages (82).

Wahl and Fouace (147) have studied the importance of this phenomenon in typing and found that lysogenization or loss of prophage may have a profound effect, so much so that the lytic group of a staphylococcus may be changed. Rountree (115), and Asheshov and Rippon (18) have shown that by lysogenization the strain 80/81 can be made to yield several different variants, among them 52/52A/80, which even by the most rigorous standards has come to be regarded as a distinct »type». Besides it seems quite possible that lysogenization may take place also in the course of nature (163), especially as it is not

* Also published in Antonie v. Leeuwenhoek 1959: 25: 237 (A. C. Ruys and J. Borst).

uncommon that two different staphylococcus strains grow side by side at the same site for some time.

The action of lysogenicity does not however seem to be so detrimental to the practice of typing as might be expected; on the contrary, that it need not cause concern as a source of error is shown by the very good correlation of phage patterns with epidemiological facts (156). Perhaps it may, however, be held responsible for the frequent occurrence of NT strains in mixed cultures (21).

— *Mixed Cultures.* In typing it is advisable to start from pure cultures (26). In clinical material, however, two different staphylococcus strains may be found growing side by side. If this escapes notice, and the typing is done from several colonies together, an atypical result may be obtained, usually in the form of growth of the other strain in plaques of the other. Unless this possibility is borne in mind a result like this is easily read as non-typable, and the matter is not cleared until single colonies are tested. There is another drawback in using mixed cultures for typing: these conditions are eminently favourable for the temperate phage carried by the one strain to lysogenize the other and thus alter its typing pattern.

RESULTS OBTAINED WITH THE TYPING TECHNIQUES

EPIDEMIOLOGY OF STAPHYLOCOCCI

Advances in typing techniques have exercised a stimulating influence on a great deal of research work (for literature, see 12—13).

Numerous hospital epidemics have been traced back to their source, which in each stricken locality has been of immediate practical value: stamping out of the epidemic has been made possible by the elimination of the source or the carriers of the epidemic strain (*e.g.* 133, 132).

It has been possible to throw light on the dissemination of staphylococcus infection. For a long time the big problem has been whether the spread of the infection is mainly due to the infected patients or to the healthy carriers among the personnel. In certain epidemics the epidemic strain has been traced back to

a single nasal carrier in the personnel, and by transferring him or her elsewhere an end has been promptly put to the epidemic. Often, but not always (133, 128), the carrier responsible for the epidemic has turned out to have a staphylococcus infection, an abscess (89), sinusitis (132), etc. at the same time. However, in the great majority of epidemics studied the role of a carrier has not been thus clear; either there have been several carriers of the same strain or the strain has been found to occur in the personnel only accidentally (40, 126).

Hare & *al.*, investigating the ways in which the carrier of staphylococci is likely to disseminate the bacteria, has found that an indirect route via nose-skin-clothes-friction in movement *etc.*-air-dust, is the most important, and that significant numbers of bacteria are actually thus released into the air (62). The chief carriers are the nasal ones, who are likewise the most likely to carry staphylococci on the skin; they also constitute the largest group of carriers. The state of being a throat carrier may also be constant without there being an attendant nasal carriership (142). Auto-infection certainly partakes in the causation of staphylococcal infections, which will be easily understood when one considers the high carrier rate of the staphylococcus and its easy spread from the nose to the skin. In the case of superficial infections of the skin this mode of infection is as good as verified (139, 137), though part of cases in which the same strain has been isolated from the skin and the nose may be due to bacteria transmitted the other way round, that is, from the skin to the nose. In hospital conditions these infections are not the conspicuous feature; on the contrary, the infecting strains consist of those few hospital strains which abound in the surroundings of the patient but were almost nonexistent on admission, and the establishment of which is favoured by the antibiotic-treatment of the patient.

When the appearance and spread of different strains of staphylococci were observed in a newly-opened surgical department, it was found that the patients were instrumental in the transmission to a far greater extent than the personnel (129). Of course this does not at all exclude the role of the personnel as passive transmitters of bacteria between patients. This role is perhaps particularly important in new-born nurseries,

where it is often impossible for the nurses to have time for observing every principle of barrier nursing and where, in particular, the same staphylococcus has been found to spread among a large number of the infants, though it is but occasionally discovered in the nurses (15, 90, 42).

The new-born presents a rewarding object of study in matters concerning microbial colonization. The speed with which their bacterial flora establishes itself is quite striking; and the staphylococcus seems to be among the most efficient colonizers during the first days after birth, in home-born (44, 66) and hospital-born (44, 65) infants both. In the latter case the role of the infant's mother as a source of infection is a minor one, that of the nurses greater, and that of the environment perhaps the major one (15, 90, 42, 87). Instead, it is the mother after childbirth that is the recipient of staphylococci from the infant (46, 68, 92). Staphylococcus epidemics of infants are often attended with cases of mastitis caused by the same strain in mothers (92). Even other members of the family may be infected, and after considerable time has elapsed since the baby was discharged from the hospital (110, 127, 153).

All carriers of staphylococci are not equally dangerous spreaders of infection. Marked individual differences in the spreading capacity of bacteria in the surroundings are known to exist, in the first place (61). These differences are not accounted for by the differences in the numbers of bacteria being carried. Secondly, the ability of a staphylococcus to colonize must be kept apart from its capacity to cause clinical infections. A strain may have spread among a considerable proportion of the patients in a ward without causing any signs of infection, while another strain, though less widely spread, is almost alone responsible for the infections (105). It is generally believed, on epidemiological grounds, that strains from septic lesions are more virulent than those from healthy carriers (155, 25). Thus a single severe infection may give raise to a number of others in the vicinity. However, a high carrier rate both among patients and among personnel tends to increase the danger of infection (64).

The current mode of phage typing has been in quite extensive use in different parts of the world for 7 years. During

this period the epidemiological behaviour of *Staphylococcus aureus* has proved surprisingly similar even in regions widely apart. The same types recur everywhere and the majority of staphylococci has always been sensitive to the same phages. A common feature has also been the common association of resistance to antibiotics, virulence, and certain nosological characteristics with the same types or groups of staphylococci.

Also in Scandinavia and Finland the staphylococcus has been subjected to investigations. Serological typing has been used by Andersen in Denmark (16), Oeding in Norway (96—99, 133), and Löfkvist & *al.* in Sweden (81,80); phage typing has been employed on a large scale by Vogelsang in Norway (140—145, 111), and by Wallmark in Sweden (149—152, 77). In Finland antibiograms have been presented (102, 17, 109), and a few materials examined by means of phage typing have been published (84, 108, 135, 86).

The increased and more accurate knowledge of staphylococcus infections which has been gained during the last few years has enabled us to plan suitable measures for preventing staphylococcus infections in hospitals. At the same time, the inadequacy has been shown, in this respect, of methods, notably prophylaxis and therapy with antibiotics, that had proved so effective in combatting nosocomial infections caused by hemolytic streptococci (155). Because the danger of »house» staphylococci looms large in most hospitals, preventive action has been taken and, if an epidemic has reached threatening proportions, several conceivably adjuvant methods of defence chosen.

Although temporarily good results have been obtained in this way, the policy has had the unfortunate consequence that the value of the different preventive measures is not still fully known. Thus the battle has to be fought on unduly many fields. In very recent years several authoritative recommendations on the prevention of hospital infections have been drawn up, *e.g.* in United States (7), in Great Britain (8) and also in Sweden (9). It is universally agreed that a Committee on Infections invested with sufficient authority in each hospital together with an efficient system of reporting infections constitutes an essential part of the preventive measures. This Committee would be

charged with the duty of devising local system of investigation and control. The necessity of restrictivity in the use of antibiotics is also generally recognized. At the same time the highest degree of effectiveness and expediency must be striven after in the treatment of established infections, which presupposes a knowledge of the value of different antibiotics and their combinations in staphylococcus infections in general and in the local conditions in particular. Some articles on the subject of antibiotic treatment of staphylococcal infections are Finland's review (49) and the original work of Jawetz (70) as well as that of Bunn & *al.* (34).

ORIGIN OF ANTIBIOTIC RESISTANT STAPHYLOCOCCI

It has been found that in each hospital infections are caused and susceptible persons attacked by comparatively few strains of staphylococci. These are the so-called epidemic strains, peculiar to each individual hospital and almost without exception specifically resistant to those anti-microbial drugs which are the most generally used in the hospital (*e.g.* 79, 83, 167).

By means of typing it has been possible to demonstrate that, what happened when a patient under antibiotic treatment is attacked by bacteria resistant to the antibiotic, is usually not a mutation during treatment followed by a selection; instead, the sensitive bacteria were replaced by resistant strains already present in the hospital environment (119, 20). For this reason drug-resistant staphylococci isolated from the infections of hospital patients are often similarly resistant to different antibiotics, irrespective of which drugs have been used in the treatment.

When a mutation to resistance happens during antibiotic treatment, which then selects for the resistant mutant, the phage pattern usually remains the same as it was before treatment. Such cases are known to have happened but so rarely that they have been considered to deserve a separate description (107, 119, 21, 23, 54, 167).

Except by mutation, new resistant strains of staphylococci may arise through transduction by phage (93, 112), but no in-

stances are recorded of its having happened naturally. The transduction may also involve a change in the typing pattern of the staphylococcus.

That antibiotic-resistant hospital strains are few inside a hospital can be accounted for by the fact that mutations to resistance are comparatively few, but a resistant mutant once established finds circumstances in a hospital favourable to survival and propagation. Stranger is the fact that, by and large, antibiotic-resistant staphylococcal types encountered in different countries are likewise relatively few (21, 120, 14, 158). Resistance to antibiotics was first observed in group III, much more slowly in group I, and all strains of group II were sensitive for a long time. And it is to group III, or to the type 80, group I (116), that the greater part of strains exhibiting multiple resistance to antibiotics belong at present. It has been suggested as a plausible explanation that staphylococci belonging to group III may be characterized by a higher mutation rate (21, 69), an assumption which is not wholly without experimental support (24). An alternative possibility might be an eminent capacity of group III staphylococci for establishing themselves as hospital strains.

VIRULENCE OF STAPHYLOCOCCI

By means of phage typing it has been possible to establish a more selective correlation between the virulence of a staphylococcus and laboratory tests than with the aid of former »virulence factors» such as coagulase, phosphatase, hemolysins, *etc.* (25). Type 80, or 80/81 (117, 127), which is decidedly more virulent than most staphylococci has been discovered. Since its appearance in 1954 or thereabouts it has gained notoriety all over the world as the cause of serious epidemics, especially in maternity hospitals (116, 157). The clinical picture also differs from that which is seen in the case of the usual staphylococcus infection, the distinguishing feature consisting in the severity and the deep-seated abscesses (116, 166). Hospital epidemics due to this strain are characterized by, among other things, the numerous infections in the personnel (166).

A survey of staphylococcal epidemics reveals differences in

virulence between various strains, it is true, yet no one strain has proved so consistently more virulent all over the world that a given carrier could be labelled as exceptionally dangerous because of the type carried. In local circumstances, instead, when the current epidemiological types and the frequency of their involvement in clinical infections are known, it is possible to set apart dangerous carriers (157).

ASSOCIATION OF PHAGE TYPES WITH NOSOLOGICAL ENTITIES

There has been much search for associations between given phage types and given pictures of diseases caused by the staphylococcus. In this respect phage typing has generally proved disappointing. There are a few exceptions, however, of which the most notable is the occurrence of phage type 71, in itself quite rare, in conjunction with impetigo contagiosa (101, 134) and certain other vesicular skin infections (100).*

Infections caused by phage type 80, besides being unusually serious, show clinical peculiarities (p. 23).

All other instances of correlation between phage type and clinical picture that have been observed are merely cases of differences in frequency distribution of the broad phage groups in different infections (162, 134, 14).

Group III is responsible for epidemics of food poisoning (121, 162). Its preponderance in infections of surgical wounds and in enteritis following antibiotic therapy is an established fact; the sole reason for this may be, however, that antibiotic-resistant hospital strains as a rule belong to this group. Now that antibiotic-resistant strains of group I are on the increase it will be interesting to observe whether this ratio will be changed. The question of especial interest is whether strains of group I will be able to cause staphylococcal enteritis, as it has been assumed

* This is an intriguing finding for the additional reason that it was in impetigo and pemphigus neonatorum that the presence of a distinctive strain different from the usual Staphylococcus aureus had been suspected for a long time (48, 131), and because staphylococci isolated from impetigo differ from the rest with regard to certain cultural characteristics, too (101).

that the capability to produce enterotoxin (which in the case of food poisoning has been demonstrated beyond doubt to be characteristic of group III strains only) might be connected with the etiology of membraneous enteritis (96).

An excess of group II staphylococci — of other strains besides type 71 — in skin diseases has also been ascertained by several authors (114, 151, 134).

THE PROJECT

The chief purpose of the present investigation has been

— to present a cross section of the antibiotic-sensitivity and phage types of staphylococci *(Staphylococcus aureus)* occurring in Helsinki.

— in this way to find out to what extent cross infection by staphylococci is taking place in Helsinki hospitals (representative of various hospital types).

— to study whether differences are to be found between the various types in virulence or some other characteristics, perhaps; and making particular efforts to find out about the disease-inciting power of staphylococci that spread through hospital cross-infection.

— to create a material for comparison with a view to subsequent researches

a) in the same material, with the purpose of determining changes in the situation,

b) in other hospitals,

c) in definite disease groups.

The material that had to be gathered for the purpose seemed to enable light to be shed on some matters concerning the nature and significance of hospital cross-infection, namely

— the occurrence of staphylococci in materials consisting of various specimens, with special reference to their possible pathogenic significance.

— the existence and stability of staphylococcal carrier state.

— spreading of staphylococci in non-hospital communities.

METHODS

COLLECTION OF SPECIMENS

The specimens were usually taken with a dry swab from the pharynx, from one nostril (anterior part), from the skin, from the breasts by squeezing out a few droplets of milk, from the vagina, or from the infected area. The swab was placed into a dry test tube, in which it was carried to the laboratory. If there was plenty of pus, it was dispatched as such in a tube. Expectoration, feces and urine were likewise brought as such in glass jars or tubes. Collection of the specimens was done by the nurses of the several wards, for which reason a considerable variety of techniques was unavoidable. The time before culturing which the specimens took to reach the laboratory ranged from a few to 24 hours. In hospital B the specimens were immediately cultured on blood plates at the bedside.

ISOLATION OF STAPHYLOCOCCUS AUREUS

All specimens were cultured primarily on blood agar plates, after which in most cases the swab was inserted into a tube containing bouillon. Both the plate and the tube were incubated overnight at $+37^{\circ}$; if colonies resembling *Staphylococcus aureus* were seen on the plate, they were picked up with a platinum loop and streaked onto a sector of a phenolphthalein-phosphatechromate plate (103), referred to hereafter as phosphatase plate, and onto a quadrant of a blood agar plate; these were both incubated overnight at 37°.* Then a quantity of 2 % NH_4OH was dropped on the phosphatase plate and the phosphatase production of the growth recorded. If a definitely positive result was obtained, a colony of staphylococci was picked from the corresponding quadrant of the blood plate and inoculated in broth and in a nutrient agar stab, After having grown at $+37^{\circ}$ overnight the stabs were stored, stoppered with rubber plugs, in a refrigerator at $+4^{\circ}$. The broth culture was subjected to the following steps.

If staphylococcal colonies were not to be found on the primary plate or if they gave a negative or weak phosphatase reaction, the swab that had been incubated in broth was rubbed against a quadrant of a phosphatase

* If staphylococcal colonies of various appearances were discernible, they were each separately subcultured.

plate; after an overnight incubation the phosphatase reaction was recorded. If the growth now produced a positive reaction (weak or strong), it was subcultured on blood agar, and if it looked like staphylococci, it was further used to make a broth culture and a stab.

In testing the coagulase production of the staphylococci the slide technique (36) was employed. Only strains negative by this method were inoculated into a tube containing 0.9 ml of saline and 0.1 ml of human plasma (160). The tubes were incubated in water bath at +37° for 2 hours, whereupon they were inspected for coagulation, and negative tubes allowed to remain at room temperature for 18 hours. Strains still negative were considered coagulase-negative and excluded from the study. In the coagulase test use was made of the confluent growth of the phage typing plate (see below).

RECORDING OF GROWTH OF STAPHYLOCOCCI

If colonies of staphylococci were seen on the primary plate, the growth was rated and is denoted, depending on the number of colonies, by the sign ++ or +++. If the staphylococci could be cultured only via broth — phosphatase plate, the growth is denoted by +.

PHAGE TYPING OF STAPHYLOCOCCI

The typing was carried out according to instructions issued by The Staphylococcus Reference Laboratory (11). The agar used in the typing plates was made by Orion OY, Pharmaceutical Manufacturers and contained

Heart infusion	1000 ml
Sodium chloride	5 g
Peptone (Evans)	10 g
Agar (NZ)	11 g

The medium was delivered in 900 ml bottles, to which was added 18 ml of 1 % $CaCl_2$ solution just before pouring the plates.

If a staphylococcus was not lysed by the basic-set phages at R.T.D., the same broth culture was used the following day for typing with phages in a 1000 times higher concentration.

The phages used for typing consisted of the basic set agreed upon by the Second Meeting of the International Committee on Phage Typing of Staphylococci (10):

phages 29, 52, 52A, 79, 80	group I
3A, 3B, 3C, 55, 71	group II
6, 7, 42E, 47, 53, 54, 73, 75, 77	group III
42D	group IV
187	miscellaneous

Propagation of phages was also carried out according to the instructions of The Staph. Ref. Lab. either in broth or on agar by the freeze-and-thaw method. The phages were filtered through sintered glass filters now in use at Colindale (159) or, in some cases chiefly at the beginning of the work, through Seitz filter pads and the filtrates were tested against the new set of testing strains (Febr. 1959; 11). The phages and staphylococcal strains were obtained, through the courtesy of Dr R.E.O. Williams, from The Staph. Ref. Lab., Colindale, on October 27th, 1958 and Feb. 11th, 1959. Shortly after concluding this study, the author had occasion to type 24 control strains sent from Staphylococcus Reference Laboratory in August 1959. The same phage preparations were used as in this study. In 13 cases the typing results were identical with those obtained in Colindale; 11 cases showed minor differences, which, however, did not affect the identification of the strains.

In a part of the strains additional phages were used, *i.e.* those 5 phages recommended by Dr G. Wallmark (152). The phages which have been isolated by Dr Wallmark himself are the following:

KS 6 of lytic group I
819 and 1034 of lytic group III
155 and 166— unspecific (lysing a major part of strains of all groups).

REPORTING OF RESULTS OF PHAGE TYPING

As »types» the numbers of phages causing strong lysis were listed, a + sign being added for any weaker reactions with other phages. In the case of weak reactions only, the typing was repeated using the 1000 times higher phage concentrations in order to be sure of these weaker reactions, which thus usually proved genuine.

If a staphylococcus did not react with the phages of the basic set at RTD but did so at 1000 times stronger concentrations, the »type» was stated according to the same principles in *italics*. If even now a reaction was not obtained, the strain was considered non-typable (NT).

Reactions brought about by additional phages were not as a rule given. Results obtained with their aid will be presented in so far as they proved to be of value.

TESTING OF THE SENSITIVITY OF STAPHYLOCOCCI TO VARIOUS CHEMOTHERAPEUTIC AGENTS

The testing was performed according to Ericsson & *al.* (47) employing the same impregnated discs as he had used, obtained from the Karolinska Sjukhusets Bakteriologiska Laboratorium, Stockholm.

The following discs were used:

Penicillin (20 IU), streptomycin (50 mcg), tetracycline (50 mcg), chloramphenicol (30 mcg), erythromycin (50 mcg), oleandomycin (50 mcg),

novobiosin (50 mcg), neomycin (50 mcg), bacitracin (10 U), and sulpha (2.4 mg). In part of the tests, some or all of the following were omitted: oleandomycin, novobiosin, neomycin, and bacitracin. A suitable density of culture on the plate was obtained by diluting *c.* 1: 1000 an overnight broth culture used in the typing (one drop from a Pasteur pipette to 5 ml of 0.9 % saline).

According to Ericsson an estimation of the minimum inhibitory concentration of a chemotherapeutic agent can be based directly on the diameter of the circle of inhibition if the circumstances are kept as constant as possible. Ericsson has constructed empirical diagrams which explicitly show the concentration. When due consideration is paid to the concentration of these agents which is therapeutically attainable, bacterial strains, according to Ericsson, will be further divisible into sensitivity groups designated by him from I to IV in the order of increasing resistance. In the present investigation the sensitivity groups were recorded according to this rule, but for reasons considered useful in the treatment of the results a rougher division in the case of most agents proved helpful. In this rough classification 'resistant' includes resistance groups IV and III, and 'sensitive' groups II and I. In the case of some agents, however, the sensitivity of staphylococcus strains was so uniform that fixing the boundary between groups III and IV was considered a sensible idea so as to achieve a differentiation of practical value. Accordingly the boundary between 'sensitive' and 'resistant' was as follows:

P =	resistant	to	penicillin	in	a	concentration	over	2 IU/ml
S =	»	»	streptomycin	»	»	»	»	16 mcg/ml
T =	»	»	tetracycline	»	»	»	»	4 mcg/ml
C =	»	»	chloramphenicol	»	»	»	»	16 mcg/ml
E =	»	»	erythromycin	»	»	»	»	6 mcg/ml
O =	»	»	oleandomycin	»	»	»	»	5 mcg/ml
	»	»	novobiosin	»	»	»	»	16 mcg/ml
	»	»	neomycin	»	»	»	»	50 mcg/ml
B =	»	»	bacitracin	»	»	»	»	3 U/ml
Su =	»	»	sulphonamide	»	»	»	»	25 mg/ml

The antibiograms of the strains were thus obtained, *e.g.* PSu meaning resistant to penicillin and sulpha, sensitive to the rest.

THE SEROLOGICAL TYPING

In the case of some selected strains use was made of serological typing with the aid of slide agglutination according to Oeding (97) using sera kindly supplied by him and absorbed with strains also obtained from Dr Oeding.

SPECIMENS SUSTAINING TWO DIFFERENT STRAINS OF STAPHYLOCOCCUS AUREUS

If different-looking staphylococcal colonies were observed on the primary plate, they were subcultured and tested separately as has been described. In cases where isolation of the staphylococci was not done until after enrichment, separate colonies could not be discerned as a rule; instead, a subculture done from the phosphatase plate contained a mixture of the strains present in the original swab. On the typing plate this manifested itself as anomalous-looking typing patterns and, oftener still, in the form of separate phage plaques scattered over the entire surface of the confluent growth. In every case that aroused the slightest trace of suspicion a subculture was derived either from the original plate or from the stab culture made from the original; two or more colonies as dissimilar as could be found were subcultured in a new broth and agar tubes for further testing.

STANDARD ERROR OF A PROPORTION

was calculated from the formula

$$\sigma_a = \sqrt{\frac{a\ (100 - a)}{n}}$$

in which a is the proportion (per cent) and n is the total number of individuals, specimens or strains.

When the significance of the difference between two proportions a and b was evaluated, the difference was compared to the value.

$$\sigma_{ab} = \sqrt{\sigma^2_a + \sigma^2_b}$$

If the difference was more than three times as great as σab it was considered to be significant. The probability (P) of such a difference occurring by chance is less than 0.003.

Isolation of the staphylococci was carried out partly in the laboratory of The Children's Hospital, University of Helsinki (Dr I. Rantasalo), partly in The Department of Serology and Bacteriology, University of Helsinki (Drs T. Kosunen, R. Mäkitalo, and O. V. Renkonen) and in part in The Municipal Bacteriological Laboratory of Helsinki (the present author); the subsequent procedures were carried out chiefly in the last-mentioned laboratory; for a small part the phage typing was done and resistance determined in DSBUH, using the same phages and sensitivity discs in both and the same person (the author) for reading the results of phage typing of all strains.

MATERIAL

The main part of the material was collected from October 1958 through July 1959 from 8 Helsinki hospitals or from persons domiciling in Helsinki.

The hospitals were:

(Abbreviation used)	
A	Aurora Hospital, Departments of Pediatrics and of Contagious Diseases and Medicine
Lkl	Children's Hospital, University of Helsinki
Nkl	Women's Clinic, University of Helsinki (obstetric wards)
Kir	III Surgical Clinic, University of Helsinki
Ma	Maria Hospital, Department of Surgery
I	Department of Dermatology, University of Helsinki
Tb	University Clinic of Tuberculosis Sanatorium, Helsinki
E	Eira Hospital (a private general hospital)

The material includes about 14 000 cultures, namely

1. Nasal and/or throat swabs from

a. *699 normal adults:* 292 female and 269 male students (Yo) contacted in connection with a prophylactic mass vaccination against poliomyelitis, courtesy of Dr Marja Sirola, Student Health Center, Helsinki; 104 medical students and 34 laboratory technical assistant trainees at the beginning of their clinical studies (Med).

b. *597 normal children* under 16 years: 52 children under 1 year visiting a Child Welfare Center; 545 non-infectious children entering hospitals A and Lkl with no history of a previous visit to hospital (excluding maternity hospitals).

c. *180 members of 27 families* taken by a nurse visiting the homes.

d. *1845 inpatients* of hospitals A, Lkl, Nkl, I and Tb, on admission and/or discharge.

e. *875 members of hospital personnel* in all the above-mentioned hospitals, the greater part swabbed 3 to 7 times.

2. Swabs from healthy skin of

384 infants under 2 years on admission and during stay in hospitals A and Lkl.

3. Cultures from infected sites

a. from infections with no established causal relationship of *Staphylococcus aureus* to the infection:

feces from 389 hospital patients (children with diarrhea, hospital A)

expectoration from 153 adults with pneumonia or suspected pneumonia, on admission to hospital A.

pharyngeal specimens from 199 children with upper respiratory infection, on admission to hospital A.

b. from infections with an established relationship of *Staphylococcus aureus* to the lesion:

various kinds of *pus specimens from 845 patients,* hospitalized and non-hospitalized.

In addition to this main material there was opportunity to examine a few staphylococcus strains from elsewhere, *viz.:*

107 strains from pus from 8 other hospitals in Helsinki and 3 hospitals outside Helsinki,

100 strains from pus from the Department of Medical Microbiology, University of Turku, courtesy of Professor J. A. Grönroos, M.D., and

84 strains mainly from pus from the Laboratory of Medical Microbiology, Lappeenranta, courtesy of Dr K. Hällström.

The material from hospitals Lkl and Nkl presented in this work has been described in greater detail elsewhere (135, 86, 87).

RESULTS

OCCURRENCE OF STAPHYLOCOCCUS AUREUS

EVALUATION OF CRITERIA USED FOR RECOGNIZING STAPHYLOCOCCUS AUREUS

For the purpose of preliminary identification of *Staphylococcus aureus* in the present material phosphatase reaction was used. This criterion was chosen because of the experience acquired in clinical and bacteriological routine that hardly ever was a coagulase positive staphylococcus found that was phosphatase negative. Moreover, the culture medium used for phosphatase reaction had proved reliable producing invariably identical batches. On the contrary the plasma which is used in coagulase reaction is liable to variations in quality, and an inferior batch gives an unduly large number of negative reactions. The use of the phenolphthaleinphosphate-chromate plate has the additional advantage of being a selective medium for staphylococci. By means of a single plate a large number of staphylococcus strains can be tested.

In the present study the start was accordingly made with phosphatase positive strains, which were further tested for coagulase. It was found that *c.* 1 per cent of strains that had been isolated from the phosphatase plate as positive did not consist of *Staphylococcus aureus* but of *B. subtilis, Gaffkya tetragena* or, in some few cases, of coagulase negative staphylococci. In the case of the lastmentioned organisms the phosphatase reaction had been weak without exception.

The slide test for determining coagulase was positive in approximately 90 per cent of those strains which proved finally coagulase positive; the remaining 10 per cent were positive in the tube test. These figures are in accordance with the current opinion of the usability of these methods (36, 160). The slide

test by means of which the greater part of positive reactions are rapidly and conveniently elicited is worth while in large-scale examinations and probably in the usual routine of a clinical bacteriological laboratory as well.

THE FREQUENCY OF OCCURRENCE IN DIFFERENT MATERIALS

The frequency of *Staphylococcus aureus* in the nasal specimens of the material examined, *viz.* 42 per cent in healthy adults, tillies quite well with figures quoted in the literature (p. 11) thus in a way showing the adequacy of the isolation technique used. Had no enrichment been carried out, the number of positive specimens would have been about half that obtained by the present method, with slight variations between specimens of different types (Table 1). When the staphylococcus was iso-

TABLE 1. — PROPORTIONS OF POSITIVE SAMPLES, SAMPLES POSITIVE WITHOUT ENRICHMENT, AND SAMPLES SHOWING ABUNDANT GROWTH OF STAPHYLOCOCCI (+++).

sample	material	No. of persons examined	total positives (per cent)	positive without enrichment (per cent of persons)	+++ (per cent of persons)
nose	non-hosp. healthy adults (Yo)	561	42	10	6
nose	adults, on adm. to hosp. A	143	43	25	14
nose	children, under 16 years, on adm. to hosp. A	597	60	33	21
throat	adults, on adm. to hosp. A	143	26	12	6
throat	children, under 16 years, on adm. to hosp. A	597	54	22	10
skin	infants under 6 months, 1—4 samples from same pat. during stay in hosp. A	64	53	36	30
skin	0—2 years, 1—4 samples during stay in hosp. Lkl	320	[1])	19	17
feces	0—2 years, diarrhea patients in hosp. A	389	24	5.4	3.6
expect.	adults with pneumonia, on adm. to hosp. A	153	34	16	11

Explanations: for abbreviations see Material p. 32.

[1]) cultured without enrichment only

lated only after enrichment, it certainly had often been in the minority among other bacteria to start with. However, because the objective of the study is an epidemiological one, it was necessary that as many of the carriers of the staphylococcus should be detected as possible; for this end an effective isolation was essential. In one of the hospitals (Lkl) the enrichment was omitted and consequently a lower percentage of isolations achieved. What with there being differences in the enrichment and with the transference of specimens — let alone the mode of collection — being different in various hospitals, it was not considered possible to compare the occurrence of staphylococci in the hospitals.

From a clinical point of view, specimens producing a(n almost) pure culture of staphylococci are the most important. This is denoted by +++, and the relative number of such samples will be seen in the extreme right-hand column of Table 1.

When specimens were taken from the surface of the skin of infants, 53 per cent were shown to be carriers of staphylococci; the frequency of +++ specimens varied from 17 to 30 per cent. Large numbers of staphylococci were found in nearly 100 per cent of eczematous children, but most of the specimens marked with the +++ sign were derived from healthy children, which shows that the skin of such young children is likely to provide more favourable conditions of growth for the staphylococcus than that of adults; nevertheless, it is usually harmless to the child.

Of expectorations, which were collected on admission into hospital from 153 patients with pneumonia, 34 per cent were positive, and 11 per cent rich in staphylococci; clinically, many of the latter were considered cases of potential staphylococcus pneumonia.

Of the feces of diarrheal children under 2 years of age 24 per cent were positive, but numerous staphylococci were found to grow in only 3.6 per cent of the 389 patients; in these cases the staphylococcus may have had an etiological bearing on the disease. The samples had been taken during treatment in hospital, so the possibility of nosocomial infection cannot be disregarded.

TABLE 2. — THE OCCURRENCE OF POSITIVE AND NEGATIVE NOSE AND THROAT CULTURES IN DIFFERENT AGE GROUPS OF PATIENTS ON ADMITTANCE TO HOSPITAL A

Age group	in-fection status	No. persons in group	per cent of persons in group					
			nose + throat +	nose + throat —	nose — throat +	nose — throat —	total positives in nose	total positives in throat
under 1 year	r.i.	68	49	21	15	16	69	63
	no	69	52	12	19	17	64	71
1—3 years	p.i.	46	37	15	4.4	44	52	41
	r.i.	74	32	16	16	35	49	49
	no	82	29	21	22	28	50	51
4—6 years	p.i.	14	29	36	7.1	29	64	36
	r.i.	25	28	16	24	32	44	52
	no	37	43	30	5.4	22	73	49
7—15 years	r.i.	32	16	50	6.3	28	66	22
	no	150	45	19	14	23	63	59
adults	r.i.	61	25	21	8.2	46	46	33
	no	82	15	26	6.1	54	40	21
totals for under 16 years		597	39	20	15	26	60	54
totals for adults		143	**19**	**24**	**7**	**50**	**43**	**26**

Explanations:

r.i. = respiratory infection; p.i. = purulent infection (boils, abscesses *etc.)*; no = no infection. The adult values printed in bold type differ significantly ($P < 0.003$) from the corresponding values in the collected material of children.

Staphylococci were found to be commoner in the pharyngeal and nasal specimens of children than in those of adults, hence the resolution of the material into age groups in Table 2. The last two columns show the percentage of positive samples in the various age groups, which have been divided into further groups according as the patient had a respiratory or purulent infection or no symptoms of infection. All the specimens were taken from the patients on admission into hospital A, which makes for the greatest possible uniformity of their examination. There are no marked differences between age groups of children under 16, but the difference in pharyngeal specimens between children

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	p.i.	46	37	15	4.4	44	52	41
1—3 years	r.i.	74	32	16	16	35	49	49
	no	82	29	21	22	28	50	51
	p.i.	14	29	36	7.1	29	64	36
4—6 years	r.i.	25	28	16	24	32	44	52
	no	37	43	30	5.4	22	73	49
7—15 years	r.i.	32	16	50	6.3	28	66	22
	no	150	45	19	14	23	63	59
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and adults is significant ($P < 0.003$): children, positive specimens 54 per cent; adults, 26 per cent. The corresponding percentages for nasal samples are 60 and 43, slightly less different, that is.

The subgroups of 'Infection' and 'No Infection' do not exhibit noticeable differences except for the group of 7 to 15 years old. Here 22 per cent of children with respiratory infection have staphylococci in the pharynx as against 59 per cent of patients with no infection. The difference is significant ($P < 0.003$) in spite of the small populations; it is also in the same direction as that ascertained by other authors (p. 12).

When a survey is made of the proportion of staphylococcus carriers (nasal and/or pharyngeal) among the examined persons, a definite difference will again be found to exist between adults and children, the percentages being 50 and 74 respectively. Among those under one year of age the carrier rate is even greater, 83 per cent. These figures are high enough, but they will be higher still if samples are taken from a person repeatedly instead of once, when the true carrier rate will be approached (p. 11).

In epidemiological examinations it is customary to collect nasal specimens only. Table 2, sixth column, in which are cases of nasal specimen being — coupled with pharyngeal +, shows that thus 14 per cent of staphylococcus carriers escape notice in the case of adults. As regards children, the proportion of pharyngeal carriers appears to be slightly greater, approximately 20 per cent of all carriers. Nevertheless, the saving in labour and material seems to justify, in mass examinations, the use of nasal specimens only.

PERMANENCE OF THE CARRIER STATE

It was possible to keep the hospital personnel under observation by means of several specimens (Table 3). From 667 persons in 4 different hospitals nasal and pharyngeal specimens were collected for a minimum of 3 times during a period of 6 weeks to 6 months. About a quarter of them proved permanently negative. A half were persistent carriers, from whom as a rule the same strain with an identical phage type and antibiogram could be isolated every time; in some cases an occa-

TABLE 3. — THE CARRIER STATUS OF HOSPITAL PERSONNEL.

	No.	per cent
total No. of persons	667	100
permanent carriers of a phage type	341	51
occasional carriers	180	27
persistently negative	146	22

Explanations:

In this table are included only those members of hospital personnel whose nose and throat were cultured on three or more separate occasions at least 7 days apart. The material is derived from 4 hospitals (A, Nkl, I and E; see p. 32).

sional negative specimen cropped up, after which the same strain returned. Still another quarter consisted of what might well be called occasional carriers: either there was only one specimen positive or, more often, various phage types, different antibiograms as well as positive and negative specimens alternated with one another.

The large number of persistent carriers (and non-carriers) was particularly striking as well as the constant manner in which the same results recurred, especially in the case of those about 100 persons from whom the two specimens had been taken as many as 6 or 7 times at intervals of one week. Further evidence of the persistence of the carrier state consists in the practically equal number of permanent carriers regardless of whether the specimens were collected at two-month intervals, as was the rule in hospital A, or whether they were taken weekly or fortnightly. A constancy of results also often manifested itself in the fact that some persons were persistent nasal carriers while others were carriers in the throat only. More frequently, however, the same strain was isolated in the nose at one time and in the pharynx at another.

HOMOGENEITY OF THE STAPHYLOCOCCAL FLORA OF A PERSON

Permanent carriers among hospital personnel were quite often found to harbour two different strains continuously, of which sometimes both, often only one at a time could be isolated owing to the technique employed. For the purpose of studying the homogeneity of the staphylococcal flora of a given person

TABLE 4. — OCCURRENCE OF IDENTICAL STRAINS (ANTIBIOGRAMS AND PHAGE PATTERNS) OF STAPHYLOCOCCI IN TWO DIFFERENT SITES OF THE SAME PERSON AT THE SAME TIME

Site 1	Site 2	Material	No. of persons with **Staph. aur.** in both sites	identical strains in both sites (per cent)
throat	nose	admission to hosp. A	219	59
inf.)	nose or throat	children 0—2 years (hosp. A and Lkl)	72	83
feces	nose or throat	children 0—2 years (hosp. A)	21	67
pus (boils &c.)	nose or throat	outpatients, mainly under 16 years [1])	105	59
pus (skin inf.)	nose or throat	dermatological hosp. I and K	268	74

For abbreviations see Material p. 32.

[1]) this material is presented in detail elsewhere (85).

use could be made of material bearing a closer correspondence to the normal population, *i.e.*, patients on admission into hospital. In the 685 cases where *Staphylococcus aureus* was isolated in two different sites simultaneously (nose, pharynx, skin, feces, pus), the strains were identical in $^2/_3$ of the cases and different in $^1/_3$. The percentage distributions within each material are shown in Table 4; no marked variations were found.

Since thus often a different strain was found in the nose and in the pharynx — when as a rule one colony from each site was tested at random —, it seems very likely that in most cases the strains had both occupied each site. This would indicate that the testing of one colony only does not give sufficient information about the specimen. For practical reasons most researchers have chosen this expediency, and Wallmark in a small material has found it satisfactory (149). Testing several colonies mixed up as suggested by Wahl (3, Arbeitsbesprechung) might, however, be better though more exacting technically, for it is not always easy to detect »anomalous» results of typing (p. 18).

While the degree of homogeneity exhibited by the staphylo-

coccus population of a single pus specimen is certainly much higher, it is not absolute, either. In superficial skin infections it is comparatively usual for two different strains to be found side by side; contamination of such infection areas as these by bacteria from the surroundings readily occurs. It is possible in deep-seated lesions as well: in the present series there was one case of fatal staphylococcal sepsis, in a two-week-old infant, where two different strains (phage types *52/79/53* and 80) were alternately found in deep abscesses and in the spinal fluid, sometimes both strains in the same specimen.

TYPING OF STAPHYLOCOCCUS AUREUS

EFFECTS OF TECHNIQUE USED ON THE TYPING RESULTS

Since the enrichment method involved a broth passage of the staphylococci in mixed culture, the effect of this was tested in 64 samples rich or moderately rich in staphylococci. 1 or 2 colonies were typed both from the primary plate and after the usual enrichment. An identical typing result was obtained in 47 cases, a related pattern with one or two minor differences in 11 cases, and a different pattern (with two or more differences) in 6 cases. The minor differences consisted either in a gain or loss of one phage reaction within a group or in an alteration in the grade of phage susceptibility, *i.e.* from reaction with phage at RTD to reaction with phage at 1000 times RTD only; the direction of these changes seemed to be random. Greater differences, such as would have entailed the identification of the strain with different types, consisted in a gain or loss of several phage reactions, *viz.* from 3A to 3A/3B/3C/55/71, from 29/52/7/54 to 7, or from *79* to NT. These differences may very well be due to the acquisition or loss of a prophage during the broth passage (p. 18).

Additional evidence that use of the enrichment technique did not detract much from the reliability of results consists in the quite large number of the so-called permanent carriers discovered (p. 38) even though the origin of the strains was not known at the time of testing them.

The antibiotic sensitivity testing gave nicely consistent

TABLE 5. — THE FREQUENCIES AND ANTIBIOGRAMS OF DIFFERENT PHAGE PATTERNS AND GROUPS OF STAPHYLOCOCCI

Total	4539	antibiograms										
		sens	Su	P	PSu	PSSu	PST[1])	PSTC[1])	PSTCB[1])	PSTB[1])	ST[2])	others[3])
		26	7.3	18	14	9.3	17	4.2	1.5	0.4	0.9	1.0
29, *29*	1.23	59	18	16	3.6	1.8						
29/52, *29/52*	1.08	47	6	38	4.0	2.0						2.0
52, *52*	2.87	59	11	11	7.7	8.5	1.5		0.8			
52/52A, *52/52A*	0.60	89	3.7		7.4							
52A, *52A*	0.29	23	15	31	23	7.7						
52A/79, *52A/79*	0.15	14	14	43	29							
79, *79*	2.49	58	23	9.7	7.1	1.8						0.9
80 (RTD)	5.77		1.9	1.1	50	42	1.9					2.7
80	0.53	33	25	4.2	21	8.3	8.3					4.2
52/52A/80 (RTD)	10.40	21	7.6	2.3	27	39	1.5					1.3
52/52A/80	3.31	15	5.3	6.7	6.7	8.6	39	17				0.7
other group I	1.17	51	21	15	7.5	3.8						1.9
3A, *3A*	6.30	33	12	38	15	2.4						
71, *71*	0.55	52	8.0	16	24							
3C/55/71, &c.	1.67	45	12	30	12	1.3						
other group II	2.82	46	9.4	32	12							0.8
6/7/47/53/54/75, &c.	3.15	36	4.2	47	9.8		1.4					2.1
6/47/53/54/75, &c.	1.72	13	6.4	56	12	2.6	3.8	3.8				2.6
others with 6	0.68	39	6.4	29	13	6.4	3.2					3.2
7/47/53/54/75, &c.	2.05	7.5	6.5	25	33	23	4.3				1.1	
others with 7, not 6	1.74	28	2.5	44	14	6.3	5.1					1.3
42 E	0.04	50	50									
others with 42 E	0.38	41	12	18	5.9							23
47/53/75/77, &c. (RTD)	0.51	13		39	35	13						
47/53/73/77 weak	7.38			0.3		2.1	72	22	0.3	0.6	0.9	1.8
53 (RTD)	0.75	15	3.0	52	15		12					3.0
53	2.05	5.4	2.1	2.1	5.4	2.1	74	6.4			1.1	1.1

others with 53	0.35	31		31	31	6.2						
54, &c.	0.44	25		55	20							
73, 73	1.74	34	11	30	22		1.3					1.3
others with 73	0.15	43	14	43								
75, 75	0.18	13	13	13	50	13						
75/77	0.09	50				25						
77, 77	0.77	54	14	17	5.7	8.6						
other group III	0.57	23	3.8	31	27	7.7	7.7					
52/79/53	4.80					1.8	97	0.5				0.5
52/52A/80/7/42E/73	2.11	8.3		64	22	3.1	1.0					2.1
groups I + III (RTD)	0.97	36	18	25	16			2.3				2.3
other groups I + III (1000 × RTD)	3.61	31	6.7	34	14	2.4	7.9	2.4	0.6		0.6	1.2
groups I + II (RTD)	0.09	50	33	17								
» I + II (1000 × RTD)	0.53	54	27	12	6.9							
other mixed-group	0.66	60	13	13	13							
NT (inhibition)	8.14	0.3		0.5		0.5	54	16	16	2.5	8.7	1.4
KS6/155/166) these are below	1.06	31	11	25	14	19						
155/166) included in NT	2.14	53	27	12	6.9							
Group I	29.88	28	9.1	6.9	23	24	5.6	2.0	0.1			1.3
» II	11.35	39	11	34	14	1.5						0.2
» III	24.73	18	3.9	24	11	4.4	29	7.3	0.1	0.2	0.4	1.8
» IV	2.38	32	2.8	57	8.3		0.9					
Misc. (187)	3.15	55	20	16	2.8							5.6
Mixed groups	12.79	19	5.3	23	9.5	1.9	39	1.0	0.2		0.2	1.0
NT	15.72	24	5.6	6.9	5.7	2.4	28	9.1	9.0	2.0	5.7	1.7

Explanations: For antibiograms see Methods, page 30. Phage patterns printed in italics indicate strains typable by phage at 1000 × RTD only. See also expl. 1—2 of Table 6.

Only one strain of each phage pattern was taken of any one person; all persons studied are included. The frequencies of phage patterns and groups are given as percentages of the total number of strains. The frequencies of antibiograms are given as percentages within each phage pattern or group. The figures printed in bold type differ significantly ($P < 0.003$) from the corresponding values of the whole material.

[1]) a major part of these being associated with resistance to sulpha

[2]) a minor part being associated with resistance to bacitracin

[3]) other antibiograms: S, T, C, PT, PTSu, PC, PSC, PSSuC, PTSuC, PSTESu, SSu, SC, STC, STB, SCSu, STCB

results as a rule. Hardly ever were different sensitivity groups recorded for the same strain on repeated testings. The density of the inoculum used was found to be the main factor affecting the diameter of inhibition circles. In regard to sulpha, however, the method did not seem to be very satisfactory, and in the following little weight is placed on the sensitivity or resistance to this drug.

PHAGE TYPES AND ANTIBIOGRAMS

In order to facilitate the handling of the material, different phage patterns were combined to form »types»; the division into types that was mainly adhered to is that adopted by Williams (158). In Table 5 each phage type is represented by a single strain per person; in this way a total of 4539 strains was obtained. Among them 31 »types» were distinguished, of which 20 occurred in more than 1 per cent of the strains. The commonest type was 52/52A/80 (10 per cent in all); this was followed by NT, with inhibition by phages 79, 6, 7, 47, 53, 75 and 77 (see p. 52); 47/53/75/77 weak; 3A; and 80 (more than 5 per cent of each).

The distribution of antibiograms in the whole material shows that the common resistance combinations were the following: fully sensitive; resistant to penicillin and sulphonamides; resistant to penicillin, sulphonamides, and streptomycin; resistant to penicillin, streptomycin, and tetracycline (accompanied in most cases with resistance to sulphonamides). The number of exclusively sulpha-resistant strains was considerable; resistance to other antibiotics nearly always occurred in combination with resistance to penicillin, as has been found elsewhere, too (31). Of the tetracycline drugs only tetracycline was used; there is, according to the literature (50, 31), a complete cross resistance between it and its derivatives.

The majority of the different phage types were either fully sensitive or only resistant to penicillin and/or sulphonamides. Strains resistant to many different antibiotics, such strains, in particular, as were resistant both to penicillin, streptomycin and to tetracycline, could be found in only few phage types, among which they constituted a majority. These are the so called »hospital strains» (p. 50); see also Fig. 1.

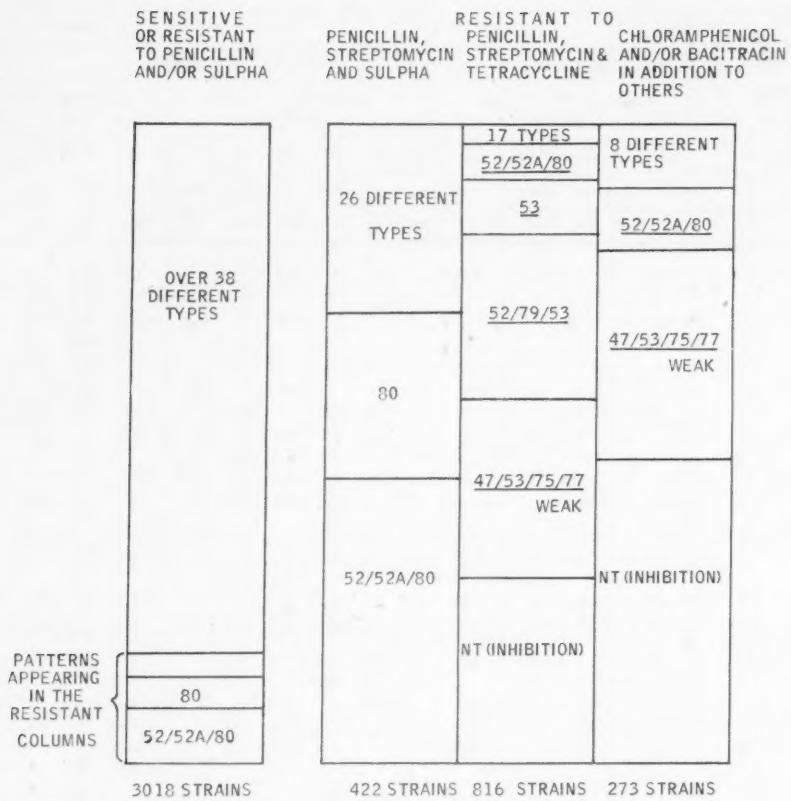

Figure 1. Chief phage types among sensitive strains (or resistant to penicillin and/or sulpha only) and among those of multiple resistance. The underlined figures in the phage types indicate typing results with phage at 1000 × RTD.

Of more interest is the distribution of phage types in mutually independent strains on the one hand and in different communities on the other. Table 6 affords a survey of the material from this viewpoint. Strains that are most likely to be unconnected are those in the first column, *i.e.* strains isolated from the nasal specimens of students. All staphylococci isolated on admission into hospital are proportionate to this normal material though not equally independent (p. 53).

Each hospital constitutes a more or less self-contained whole, of which the personnel are one component, the patients another. Children of various ages have also been assigned to due groups,

Age groups	ADULTS																
Type of material	Non-hospital				Hospital												
	«Normal» adm				Medicine							Dermat			Surg	
					Personnel			Patients				Pers	Pat		Pers	Pat
Source of material	Yo	Med	Nkl	Hki	E	A	Tb	A	Tb	A		I	I + K		Kir + Ma	
Type of samples	n	n	n	pus	n	n	n	n	n	exp	pus	n	n	pus	n	pus
Totals	234	67	104	13	91	39	50	91	146	49	82	198	746	377	96	144
29, *29* (see expl.)	3.4	1.5	1.0	7.7	1.1		2.0		3.4			1.0	2.7	2.6		0.7
29/52, *29/52*	1.3	3.0	1.0				2.0	1.1		4.1	2.4		0.8	0.3		
52, *52*	5.1	7.5	9.6	7.7			4.0	4.4	1.4		2.4	2.0	2.1	2.1	2.1	1.4
52/52A, *52/52A*	2.1	1.5	3.8									1.5	0.7	0.8		
52A, *52A*	0.4				1.1								0.8		1.0	0.7
52A/79, *52A/79*					1.1						1.2		0.3			
79, *79*	3.4		1.9		3.3		2.0	2.2		2.0		4.5	5.5	7.7	2.1	1.4
80 (RTD)		3.0	1.9	7.7	1.1	2.6	6.0	5.5	4.8	6.1	24	2.0	3.3	3.7	11	15
80	0.9		1.0					1.1	0.7	4.1	1.2		0.7	1.3		
52/52A/80 (RTD)	12	12	9.6		5.5	2.6	4.0	7.7	13	12	3.7	5.6	4.8	3.7	5.2	4.9
52/52A/80	3.0	1.5	1.9		1.1		4.0	8.8	6.2	4.1		5.1	4.7	4.0	3.1	6.3
other group I	1.3		1.9		1.1			2.2				1.0	1.2	0.8		
3A, *3A*	9.4	3.0	9.6	7.7	7.7	2.6	2.0	11	8.9	6.1	1.2	5.1	6.3	5.0	7.3	3.5
71, *71*	1.3	1.5		7.7					0.7				1.3	1.1		0.7
3C/55/71 &c.[1])	0.9		1.9	15		5.1	2.0	1.1	0.7		2.4	1.0	1.2	1.8	5.2	0.7
other group II	3.4	1.5	1.0		6.6	5.1		1.1	0.7	4.1	2.4	3.0	3.1	3.2	1.0	7.0
6/7/47/53/54/75 &c.[1])	4.7	4.5	5.8	7.7	7.7	5.1	2.0	3.3	1.4	2.0		5.1	3.0	3.4	7.3	
6/47/53/54 &c.[1])	1.7	3.0	2.9				4.0	3.3		8.2	2.4	2.5	2.2	3.2		
others with *6*			1.9		3.3		2.0					1.0	2.2	1.5		1.4
7/47/53/54/75 &c.[1])	1.7	3.0	1.0		5.5	10		3.3	1.4	4.1		1.0	2.2	2.6	4.2	1.4
others with 7, not *6*	0.9	7.5	4.8		3.3	2.6		1.1	2.1	2.0	1.2	3.5	1.2	0.5	2.1	2.1
42E &c.[1])		1.5										1.0	1.1	0.5		
47/53/75/77 &c.[1])							4.0	1.1		2.0	1.2		0.8	0.3		1.4
47/53/75/77 weak	1.7	3.0	1.9		1.1	7.7	18	2.2	2.1	4.1	15	3.0	3.6	4.2	21	17
53 (RTD), &c.[1])	2.6		1.0		3.3	2.6	2.0	1.1			1.2		2.1	1.3	1.0	2.8
53	0.9	1.5	1.0		1.1		2.0	1.1	0.7	2.0	3.7	3.5	0.2	1.1	1.0	0.7
54 &c.[1])	0.4	10										0.5		0.3		
73 &c.[1])	2.6		2.9		8.8	2.6	2.0	3.3			2.4	4.5	3.6	3.7	2.1	2.8
75 and/or 77 &c.[1])	0.9	3.0			2.2			2.2	1.4			2.5	1.2	1.1		1.4
other group III													0.1	0.3		
52/79/53			2.9				2.0	2.2	3.4	2.0	1.2		0.4	0.3	1.0	2.1
52/52A/80/7/42E/73	1.7	3.0	3.8	7.7	2.2	5.1	6.0	3.3	2.1	2.0	4.9	2.0	1.6	2.1		1.4
groups I+III (RTD)	3.0	4.5	1.9		3.3	2.6	2.0	1.1		2.0			1.3	1.1		2.1
other groups I+III (1000×RTD)	6.4	1.5	4.8		3.3	15	6.0	5.5		8.2	4.9	2.5	3.9	4.5	6.3	8.4
groups I+II (RTD)		1.5														
» I+II (1000×RTD)	0.9				3.3		2.0	1.1	3.4	8.2			0.9	0.5		
other mixed-group	2.1		1.0		2.2			1.1					1.3	0.3		
NT (inhibition)[2])			4.8		7.7	5.1	6.0	4.4	32	2.1	12	13	8.2	9.0	4.2	9.7
Group I	33	30	33	23	15	5.1	24	33	29	33	35	24	28	27	25	30
» II	15	6.0	12	31	14	13	4.0	13	11	10	6.1	9.1	12	11	14	12
» III	18	37	23	7.7	36	31	36	22	8.9	25	27	29	23	24	39	31
» IV	2.6		2.9		1.1	7.7	8.0	3.3	0.7	2.0	2.4	5.6	1.9	2.4	4.2	
Misc. (187)	6.0	3.0	1.9	7.7	1.1	5.1		2.2	0.7	6.1		4.0	6.0	5.0	1.0	
Mixed-group	14	10	14	7.7	15	23	18	14	8.9	14	11	4.6	9.3	9.2	7.3	14
NT	12	13	12	23	17	16	10	12	40	10	18	24	20	21	10	14

Explanations: For detailed description of the material and abbreviations of hospitals see page 32. Other abbreviations: adm = patients on admission to hospital; n = strains from nose or throat; exp = from expectoration; fec = strains from feces; Hki = persons living in Helsinki. Patterns printed in italics indicate strains typable by phage at 1000 × RTD only. The frequencies of different phage patterns are given as percentages of the total

CHILDREN																				ADULTS	
5 days				under 1 year					1—3 years					4—15 years							
				Non.-h		Hospital			Non.-h		Hospital			Non-h		Hospital				Hosp.	
Obstetrics																				Pediat	
Pers	Pat	Pat				Patients			adm		Patients			adm		Pat				Pers	
Nkl		Nkl		Hki		A	Lkl	A	Hki		A	A	A	Hki		A	A			A	Lkl
n	n	n	pus	n	pus	n	n	pus	n	pus	n	pus	fec	n	pus	n	pus			n	n
285	155	168	37	153	51	246	208	42	70	78	209	23	109	155	127	325	18			317	80
	0.6					0.8	0.5			1.3		4.3		1.3	0.8	2.2				1.6	
1.1	1.3	0.6		5.2	3.9	2.0	0.5		2.9	2.6	1.4		0.9	4.5	1.6	2.5				1.6	
2.1	1.9	0.6		1.3	2.0	2.0	1.0		4.3	3.8	3.8		2.7	5.2	6.3	7.4	17			1.3	3.8
0.7			2.7											0.6	0.6	0.9				0.3	
						0.4		2.4		1.3	0.5					0.3					
					2.0						0.5			1.3		0.6					
1.4	1.9	0.6	2.7	0.7	3.9	0.8					1.4		0.9	0.6	0.6	1.8				2.5	2.5
4.9	4.5	9.5		3.6	20	3.7	5.3	14		19	2.4	13	2.7		12	1.2	28			5.1	8.7
						0.8	1.4		1.4		1.4			0.6		0.6				0.3	
7.0	5.8	2.4	13	18	7.8	19	12	12	16	9	17	13	25	11	15	13	5.6			17	11
1.8	1.3	1.8		0.7	2.0	1.2	5.3		2.9	6.4	3.3		2.7	1.9	2.4	2.5				2.2	2.5
0.4			2.7	2.0		0.4				2.6	1.0			4.5	3.9	4.3	5.6			1.9	
9.8	10	4.2	2.7	5.9		7.3	3.8		16	5.1	14	8.7	10	13	3.9	11				8.2	14
						0.4			1.4	2.6	1.0				1.6					0.6	
0.7	0.6	0.6	11	1.3		0.4				6.4	0.5		0.9	1.3	6.3	2.2	5.6			2.5	
2.1	1.3	1.8				1.2	0.5	4.8	2.9	6.4	2.9	8.7	2.7	0.6	8.7	2.5				1.9	3.8
8.4	4.5	6.0	2.7	0.7		0.4	1.4		5.7		2.4	4.3	1.8	3.9	0.8	3.7				4.4	
1.8	1.9					0.4	0.5		1.4		1.9		1.8	2.6		1.5				3.5	1.2
0.4				1.3		1.2			1.4		0.5		0.9							0.6	
3.8	1.9	2.4		1.3		2.0	1.0	2.4	1.4		2.4		0.9	0.6	1.6	1.5				4.1	3.8
3.5				3.3		2.0	1.0		1.4	2.6	1.0		0.9		5.5	0.6				1.3	
										1.3	0.5			1.9		1.2					
1.1			5.4						1.4		0.5		0.9	1.3		0.9				0.6	
6.3	2.6	23	19	11	7.8	9.3	14	21	13	1.3	11	4.3	8.2	2.6	2.4	2.8				8.5	10
1.1	1.3	0.6			2.0	0.8			1.4		1.0					0.6				0.3	
1.4	5.8	2.4	5.3	3.3		5.3	13	4.8	4.3	3.8	2.4		3.7			0.6					5.0
0.7							1.9				0.5									0.3	
1.4	1.9	1.8		1.3	5.9	0.8			1.4		1.0			0.6	7.1	0.6				2.2	
1.8		0.6		0.7		0.8	1.0							1.3		0.9				1.2	1.3
							0.8							0.6		0.6					
3.9	21	22	16	14	18	14	9.4	19	1.4	2.6	3.3		9.2	0.6		0.9				3.5	6.2
3.9	3.2	0.6	2.7	1.3	5.9	1.6	1.0			2.6	1.0	4.3	0.9	0.6	1.6	1.5	17			2.5	2.5
0.7	1.3			1.3		0.4				3.8	1.0	4.3	0.9	1.3	0.8	0.6				0.3	
4.2	2.6	0.6		3.3	2.0	2.0	5.8		1.4	7.7	3.3		5.5	3.2	1.6	3.7	5.6			0.6	2.5
									1.4		1.0			0.6		0.3				0.3	
0.7															0.8					0.3	
	0.7						0.4	0.5					0.9	1.3	0.8	1.2					1.2
4.9	13	8.3	2.7	5.2	2.0	4.5	13	9.5	2.9	1.3	5.0	4.3	5.5	1.9	0.8	2.5				6.0	10
19	17	15	21	30	39	31	26	31	27	46	33	31	35	32	43	37	56			33	29
13	12	6.6	14	7.2		9.3	4.3	4.8	20	21	18	17	14	15	21	16	5.6			13	17
32	20	36	32	23	16	23	35	29	33	9	25	8.7	19	15	17	16				28	21
6.3	2.6	7.2	2.7	5.2	7.8	3.3			2.9		1.9	13	0.9	1.9	3.9	1.2				2.8	
3.2	3 2	0.6	2.7	1.3	2.0	2.8	2.4	4.8	2.9		1.9	8.7		7.7	2.4	4.9	11			1.3	3.8
14	29	25	19	21	25	19	17	19	4.3	17	9.0	8.7	17	8.1	5.6	7.9	22			9.7	13
13	16	10	8.1	13	8.0	11	16	12	10	7.7	12	13	15	20	7.0	17	5.6			11	16

in each column. In each column, a strain of any one phage pattern from a patient is recorded once only; it can, however, be met with in different columns, e.g. nose and pus from the same source.

[1]) a group of types closely related to the main pattern

[2]) a recurrent pattern with inhibition by the phage filtrates 79, 6, 7, 53, 75, 77.

since they turned out to differ considerably from the normal adult material (see also p. 54).

In the normal material, too, the commonest phage type is 52/52A/80 (12 per cent), which, unlike in the whole material, is followed by 3A (c. 9 per cent), 187, 52, and 6/7/47/53/75 (about 5 per cent each). In the whole material these are preceded by several types not found in, or comprising but a small part of, the normal material. The strains in question are usually resistant to antibiotics and strongly represented in hospital materials.

The distribution of types in the normal material certainly corresponds more closely to the occurrence of the various types in the population at large. In the normal material there is also less resistance to antibiotics than in the whole material and most of the resistant strains belong to group III (Table 7), in a smaller

TABLE 7. — STRAINS RESISTANT TO 10 CHEMOTHERAPEUTIC AGENTS IN DIFFERENT PHAGE GROUPS IN THE WHOLE MATERIAL AS CONTRASTED TO NORMAL MATERIAL (*i.e.* NON-HOSPITAL HEALTHY ADULTS' NOSE STRAINS)

	non-hospital adults (nose strains)								
	totals 234	resistant to							
		none	Su	P	S	T	C	B	E, O
Group I	81	81	9.1	16	2.6				
Group II	35	69	14	31					
Group III	42	26	12	69	9.5	12			
Group IV	6	17		83					
Misc. (187)	14	100							
Mixed groups	38	56	9.4	37					
Non typable	18	96	3.6						

	whole material (as table 5)								
	totals 4539	resistant to							
		none	Su	P	S	T	C	B	E, O
Group I	1356	28	65	62	33	8.7	2.7		0.1
Group II	515	39	27	50	1.5	0.2	0.2		
Group III	1123	18	58	78	43	39	8.0	0.3	0.1
Group IV	108	32	12	66	0.9	0.9			
Misc. (187)	143	55	23	19		0.7	4.9		
Mixed groups	580	19	57	75	43	41	1.7	0.2	
Non typable	714	24	37	56	57	55	19	11	

Explanations: The figures are percentages within each phage group. Su = Sulpha, P = Penicillin, S = Streptomycin, T = Tetracycline, C = Chloramphenicol, B = Bacitracin, E = Erythromycin, O = Oleandomycin. Strains resistant to Novobiocin or Neomycin were not encountered.

measure to group I, which wholly accords with the general opinion of how staphylococci of the different groups tend to give rise to antibiotic-resistant varieties. In the whole material, in which resistance is more common and the proportion of interdependent strains higher, this association between resistance and the groups is partially concealed: the high resistance rates of mixed-group and non-typable strains shown in Table 7 are mainly due to the prevalence of a single resistant type in certain hospitals.

USEFULNESS OF THE ADDITIONAL PHAGES

Phages 155 and 166 are very much alike, they almost always lyse the same strains. These phages are known to be unspecific (151), which they proved to be in this investigation, too, lysing most strains of groups I and II and many of group III. Strains only lysed by them were met with in 2.14 per cent of all strains (Table 5), which means that these phages ought to help differentiate a considerable part (14 %) of NT strains. In this material these phages provided not a little help by giving additional support in diagnosing one of the main hospital strains (p. 50).

Phage KS6 alone (or in combination with 155 and 166), which is a group I strain, lyses 1.06 per cent of all strains. Most important it has been in the differentiation of strains within the type 52/52A/80, numerically the largest type in this material and of epidemiological interest as well. Within this type the strains are occasionally more weakly lysed by phage 52A, sometimes an equal degree of lysis is caused by all three, but this is by no means a reliable criterion for use in strain differentiation. Table 8 shows that lysis by phage KS6 divides this type in two parts of almost equal size. In comparison with the occurrence

TABLE 8. — PHAGE KS6 DIFFERENTIATING THE PHAGE PATTERN 52/52A/80

	per cent	
	lysed by KS6	not lysed by KS6
Of 258 Finnish strains »52/52A/80»	56	44
Of 137 Swedish strains »52/52A/80» (152)	77	23

rates quoted by Wallmark (152) the present material contains less 52/52A/80/KS6. In hospitals examined, none of which harboured these as their epidemic strains, the distribution of 52/52A/80/KS6 and 52/52A/80 was very similar to that in the whole material. Strains of type 80, too, are lysed by phage KS6 in nearly 100 per cent of cases.

Phages 819 and 1034 of group III did not prove very useful in this material. Only few strains lysed by 1034 alone and one by 819 alone were encountered. About the same number of group III strains is lysed by both, by neither, and by 1034. Within this group, however, a very satisfactory degree of strain differentiation is already obtained by means of the basic-set phages.

DEMONSTRATION OF HOSPITAL STRAINS

Some few antibiotic-resistant phage types in the material occur with such frequency as cannot be accounted for except by cross-infection. On grounds stated below the most typical hospital strains in the material appear to be these four: NT, with inhibition by phages 79, 6, 7, 47, 53, 75, and 77; *47/53/75/77* weak; *52/79/53; 53.*

1. In the material these strains were found in 2.6 per cent of the undergraduates, in 18.4 per cent of hospital personnel, in 21.3 per cent of persons with a history of hospitalization, and in 30 per cent of children under 3 years of age (see also p. 54).

2. Of strains belonging to these types, 99, 98, 98, and 83 per cent respectively exhibit multiple resistance, *i.e.*, to penicillin, streptomycin and tetracycline at the least.

3. Of staphylococci of multiple resistance, as is shown in Fig. 1, p. 45, 94 per cent belong to these four types.

In addition to those already mentioned, approximately one half of three more types, *viz. 52/52A/80,* 52/52A/80 and 80, are resistant to at least penicillin and streptomycin. In the case of these types, however, an accumulation in hospital materials is more difficult to notice because a considerable part of the strains are sensitive. Although the frequency of their occurrence in any of the hospitals is not significantly higher than in the normal material (in part owing to the paucity of the normal material), the occurrence of the resistant type *52/52A/80* in

certain wards of one hospital has been demonstrated earlier (86) as well as the frequent occurrence of 52/52A/80 in one of the two large lying-in hospitals in Helsinki (108).

Below, here and there the first-mentioned four types will be treated as special hospital strains because they are quite well suited to illustrate the occurrence of the most resistant strains in different sections of the material. Nevertheless it goes without saying that a large proportion of the last three could be thus called with equal reason, and that in other hospitals there may be different »house» strains, commoner than these.

In the case of the hospital strains a departure was made from Williams's classification (158), in which none of these »types» occurs. The deviation is based on epidemiological evidence: when a group of closely related patterns kept recurring in the typing material, when they all exhibited a similar antibiogram, clearly different from that of others, and when it was possible to type several strains derived from the same person at different times, the modal pattern and the range of variation could be worked out so as to establish the type as a distinct entity and to exclude the irrelevant patterns even though the difference might amount to less than two strong reactions (see also p. 17). Indeed, by adherence to conventional principles of type differentiation it would have been impossible to distinguish from one another even three of the commonest hospital strains showing no strong reactions and a common reaction with phage 53.

Type *52/79/53* produced this pattern very consistently, always with the phages at 1000 times RTD only. One or other of these phages perhaps reacted less strongly than the rest; the strongest lysis was usually caused by 53. It sometimes showed a weaker lysis by phage 77, and certainly is identical with 52/79/53/77 of Rantasalo (108). Likewise, *53* reacted only at 1000 times RTD with phage 53, and never with other phages. The greatest variation was shown by *47/53/75/77* weak, partly because the lysis was extremely weak as a rule, often only a few plaques with phage at 1000 times RTD. Identification of this type was aided by its lysis by phages 155/166 at RTD: this lysis differed from that of most other strains by these phages in that much secondary growth appeared on the lysed area. The different patterns which have been ascribed to this type are shown

in Table 9; they mean lysis (weak) by phages 6, 7, 47, 53, 54, 73, 75, 77, sometimes also by 29, 52, 52A, 79, 80, or 3A. Of the additional phages also KS 6 at 1000 times RTD has often given a lysis. Another characteristic consists in the fact that phage 73 has lysed the type only seldom, and never more strongly than the other phages have done.

The most precarious step, it seemed, was the assumption that the nontypable strain would be another »type». Yet the pattern of inhibition by phages 79, 6, 7, 47, 53, 75, and 77, or by several of these was very constant when different strains from a person were typed, and the antibiogram was highly characteristic as well. While the other hospital strains are usually resistant to sulpha, these NT strains are not, despite their resistance to 2, 3, 4, or 5 antibiotic substances. The inhibition pattern was not always equally clear-cut, and then the strain got lumped in with the mass of NT strains; among these, judging by the similarity of antibiograms, there were strains that rather belong to the hospital type NT with inhibition. In any case they were not many.

Epidemiological circumstantialities seem to warrant the separation of *52/52A/80* from 52/52A/80; in no instance were the two types found in a person but that their antibiograms were dissimilar. At the same time a number of patterns closely related to *52/52A/80* had to be placed among mixed groups, when there were additional reactions with phages 7, 47, 53, 75 and/or 77. If, as often happened, those group III phages which caused lysis together with the three group I phages 52, 52A and 80 were 7, 42E and 73, a different type seems to have been involved, for in the last-mentioned pattern strains of multiple resistance are virtually non-existent.

SEROLOGICAL TYPING OF THE HOSPITAL STRAINS

Additional support to the identification of the hospital strains was provided by serological typing. Ten strains that had been classified as type NT with inhibition reactions were typed and found to be of the serological type *abcehk* or *abcek*, group 4. The same serological type was revealed in the case of 12 strains from 9 different hospitals, regarded as type *47/53/75/77* weak.

TABLE 9. — DIFFERENT PATTERNS WITHIN TYPE *47/53/75/77* WEAK

6/7/47/53/75/77, &c.	4.9 %
7/47/53/75/77, &c., not 6	15 %
47/53/75/77, &c., not 6 or 7	41 %
53/75/77, &c., not 6, 7, 47	12 %
54/75/77, &c., not 6, 7, 47, 53	4.9 %
75/77, not 6, 7, 47, 53, 54	5.3 %
77, not 6, 7, 47, 53, 54, 75	7.0 %
KS6, no phages of basic set	2.1 %
155/166, no other phages	3.8 %
patterns containing phage *29* (+ the group III phages)	1.1 %
» » phages *52*, *52A* or *79* (— » —)	0.9 %
» » phage *80* (— » —)	1.3 %
» » phage *3A* (— » —)	0.2 %
modal pattern *47/53/75/77* weak or *47/53/75/77/KS6/155/166* weak when additional phages are used	471 strains
belonging to group III	97 %
mixed groups	3.4 %
mixed groups if phage KS6 is respected	37 %

Explanations: when phage numbers are printed in italics, the reaction was obtained with phage at 1000 × RTD only.
&c. indicating a group of types closely related to the main pattern.

Four strains of type *52/79/53* were serologically *abc(h)i*, group 3; 7 strains of type *53* in serological typing gave the patterns *e*, *(a) e*, *(c)e(h)*, *ei*, and *aei*, group 1.

As serological typing is a time-consuming method, it was not applied further. Owing to comparative inexperience the reactions often were not definite enough to be relied on until repeated.

EPIDEMIOLOGICAL IMPLICATIONS

THE NORMAL MATERIAL

As a normal material many workers have used staphylococci isolated from patients with no infection on admission into hospital. A comparison of such strains derived from the different hospitals with strains isolated from the students outside any hospital (Table 10) will show that among the former both resistance to antibiotics and actual hospital types are commoner. It is probably true that of patients admitted for treatment a higher proportion than of the population at large have come into contact with a hospital before, either as inpatients or outpatients

TABLE 10. — A COMPARISON OF DIFFERENT «NON-HOSPITAL« MATERIALS OF ADULTS' NOSE OR THROAT STRAINS

	1.	2.	3.	4.	5.	
	students	med. students	on admission to hospital			total for 2+3+4+5
	Yo	Med	Nkl	A	I	
total	234	67	104	42	245	458
sensitive strains %	67	51	54	38	35	42
strains resistant to Penicillin	30	49	45	53	40	44
— » — Streptomycin	3.0	10	15	12	19	16
— » — Tetracycline	2.1	4.5	12	9.5	16	13
— » — Chloramphenicol	0.0	0.0	1.0	7.2	5.3	3.7
»Hospital strains» total %	2.6	3.0	12	7.2	14	11
NT (inhibition)			4.8	7.2	10	7.2
47/53/75/77 weak	1.7	3.0	2.9		4.1	3.3
52/79/53			2.9			0.7
53	0.9		1.0			0.2

For abbreviations see Material p. 32.
For hospital strains see p. 50.

(or have been given antibiotics at home, which, however, must be of minor importance because the resistant strains in this material, too, consist of those few known hospital strains). In the case of the hospital for skin diseases this is known to be true for certain, because a large proportion of the patients are sufferers from chronic complaints, who present themselves for repeated periods of treatment at hospital and between times are staying at home. Likewise, in medical students antibiotic-resistant strains are commoner than in other undergraduates. It may be argued of course that young university students are healthier than the population in general and thus less likely to be brought into contact with hospital conditions. At any rate this group of 234 strains appears, best of all the material available, to meet the requirements a normal material is expected to fulfil.

STAPHYLOCOCCI IN DIFFERENT AGE GROUPS

Different occurrence rates of staphylococci were observed between various age groups (p. 37). Even greater differences will be found in the distribution of antibiotic-resistant strains

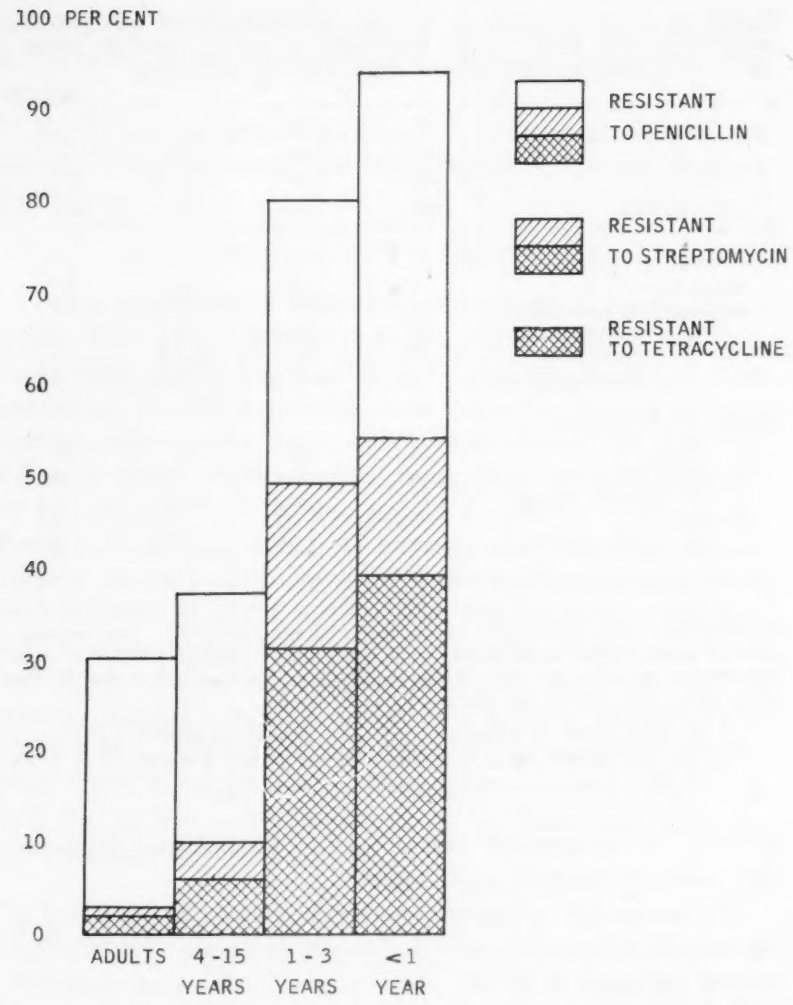

Figure 2. Percentages of antibiotic resistant staphylococci in different age groups.

according to age. In default of a good normal child-material, patients' on-admission strains must be used instead (Table 11, Fig. 2). The used material is derived in its entirety from one and the same hospital (A), where the corresponding case histories were accessible as well. Thus it was possible to include in the material only such patients as had not received treatment in a hospital previously. In the case of the infants this is of course a

TABLE 11. — A COMPARISON OF THE OCCURRENCE OF ANTIBIOTIC RESISTANCE AND SOME PHAGE PATTERNS IN NASAL STRAINS FROM DIFFERENT AGE GROUPS (NON-HOSPITAL SAMPLES)

	Age groups			
	under 1 year [1]	years 1—3 [1]	years 4—15 [1]	adults Yo
total strains	153	70	155	234
Sensitivity:				
sensitive	**6.0**	**18**	**60**	**67**
resistant to penicillin	**94**	**80**	**37**	**30**
— » — streptomycin	**54**	**49**	**9.7**	**3.0**
— » — tetracycline	**39**	**31**	**5.8**	**2.1**
— » — chloramphenicol	2.0	7.1	1.3	0.0
Special phage patterns:				
1. 52/52A/80	18	16	11	12
2. *52/79/53*	**14**	1.4	0.6	0.0
3. *47/53/75/77* weak	11	13	2.6	1.7
4. *53*	3.3	4.3	2.4	0.9
5. NT (inhibition) [2]	5.2	2.9	1.9	0.0
The 4 hospital strains (2.—5.)	**34**	**21**	7.5	2.6

Explanations: For description of the material see also p. 32. All frequencies are given in per cent within each age group. Figures printed in bold type differ significantly ($P < 0.003$) from the corresponding value in the adult age group and in the age group 4—15 years.

[1] on admittance to hospital A or from children's welfare center.
[2] a phage pattern with inhibition by the phage filtrates 79, 6, 7, 53, 75, 77.

question of construction seeing that in Helsinki all deliveries take place in hospital confinement.

The proportion of antibiotic-resistant strains in the group of the 4 to 15 years old is not significantly in excess of that in the normal material of adults. This in part shows that children's strains on admission constitute a fairly suitable normal material. On the contrary, only about one fifth of all strains in the age group 1 to 3 years are sensitive ($^2/_3$ in adults), the proportion of strains resistant to various antibiotics being correspondingly higher. The trend continues in those less than one year old, with a drop in the percentage of sensitive strains to 6, while 54 per cent are resistant to streptomycin and 39 per cent to tetracycline.

Most strains of multiple resistance that were met with in the youngest age groups can be related to those hospital strains

which occur in Helsinki maternities — a fact strongly pointing to the instrumentality of lying-in hospitals in propagating these strains.

Since there are such big differences between the various age groups, these have to be dealt with separately in the following paragraphs.

STAPHYLOCOCCI IN FAMILIES

When staphylococci isolated from members of families were typed, each family appeared to have a staphylococcus type of its own, which could be found in several of its members. The occurrence of such a familial strain was most distinct in large families; for instance, seven members in a family of eight had the same strain. These familial types were generally sensitive or penicillin-resistant strains and of a different type in each family, which shuts out from consideration the possibility of hospital strains having cumulated in the families. Of 24 families each an average of 3.4 carriers (not less than two, however) were examined. The total number of strains isolated from them was 96. In each of these families there were, on an average, 2.9 persons carrying a strain identical to a strain of somebody else in the family. By way of comparison, 82 unconnected persons, from whom 96 staphylococcal strains were isolated, were similarly grouped to make 24 »families». In these »families» the corresponding figure was 0.7.

THE EFFECT OF HOSPITALIZATION ON THE CHARACTER OF THE STAPHYLOCOCCUS FLORA OF THE PATIENT

A comparison of staphylococcus strains isolated from the patients on admission and later, at discharge or during confinement (Table 12), reveals an increase in both hospital strains and resistant strains in general. The increase is not always very marked; and suitable materials for this comparison are rather small. In the hospital for skin diseases, for example, the patients are harbouring plenty of typical hospital strains at the time of admission already. The most graphic illustration of this change is provided by the maternity hospital, where during 5 days' confinement the mothers' staphylococcus flora altered thus: 45

TABLE 12. — THE ACQUISITION IN DIFFERENT HOSPITALS OF PENICILLIN-RESISTANT (P) STRAINS AND OF THE FOUR »HOSPITAL STRAINS«: NT WITH INHIBITION, *47/53/75/77* WEAK, *52/79/53* AND *53*.

The hospital materials	total carriers	per cent of carr. P	4 »hospital strains« (per cent of strains)				
			together	NT (inhib.)	*47/53/75/77* w	*52/79/53*	*53*
Medicine (A)							
on admission	42	53	7.2	7.2			
at discharge	78	64	10	4.4	2.2	2.2	1.1
Dermatology (I)							
on admission	245	40	14	10	4.1		
at discharge	483	55	12	8.2	3.6	0.4	0.3
Obstetrics (Nkl)							
on admission	104	45	12	4.8	2.9	2.9	1.0
at discharge	121	75	42	13	2.6	21	5.8
Pediatrics							
4—15 years (A)							
on admission	155	37	7.5	1.9	2.6	0.6	2.4
at discharge	236	47	6.4	1.9	2.8	0.9	0.8
1—3 years (A)							
on admission	70	80	22	2.9	13	1.4	4.3
at discharge	165	79	22	5.0	11	3.3	2.4
under 2 years (Lkl)							
on admission	56	71	21	3.6	3.6	5.4	8.9
at discharge	163	90	50	13	14	9.4	13
under 1 year (A)							
on admission	99	86	36	6.0	10	16	4.0
at discharge	140	89	33	4.5	9.3	14	5.3

Explanations: Frequencies at discharge printed in bold type differ significantly (P 0.003) from the corresponding values on admission. For description of material see page 32; for hospital strains see page 50.

per cent had penicillin-resistant strains on admission, 75 per cent at discharge; hospital strains, 12 per cent on admission, 42 per cent at discharge.

In infants, changes are difficult to detect because of the abundance, which is being carried on admission already, of resistant strains and those hospital strains which are on the prowl inside the hospital. In one of the two children's hospitals (Lkl), however, the increase in hospital strains is significant ($P < 0.003$). In this hospital, the admission strains are less resistant, possibly due to the fact that many patients come from the countryside.

STAPHYLOCOCCI IN THE DIFFERENT HOSPITALS

From Fig. 3 it will be seen that staphylococci isolated from the nose or pharynx of hospitalized patients, those from hospital staff as well as strains isolated from purulent infections of the patients were about equally resistant to penicillin, streptomycin and tetracycline, and definitely more so than those derived from the normal material. Table 13 presents these materials in

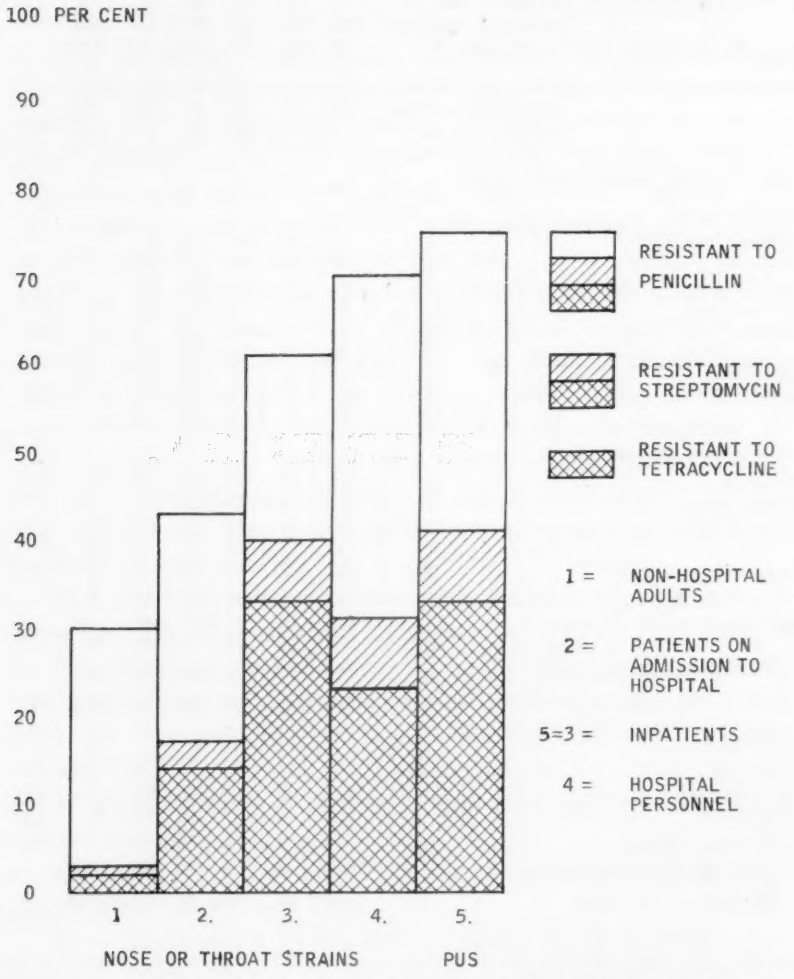

Figure 3. Percentages of antibiotic resistant staphylococci in various hospital and non-hospital materials (adults).

TABLE 13. — PERCENTAGES OF STAPHYLOCOCCAL CARRIERS CARRYING STRAINS RESISTANT TO VARIOUS ANTIBIOTICS IN DIFFERENT HOSPITAL AND NON-HOSPITAL MATERIALS.

		total carriers	strains resistant to						
Type of material			sens	P	S	T	C	B	E, O
1. Non-hospital adults, noses	Yo	234	67	30	3.0	2.1			
2. Hospital personnel, noses		690	29	70	31	23	6.0	2.5	0.3
3. Inpatients noses or throats, adults		936	37	61	40	33	12	4.0	
4. Inpatients, pus, adults		466	24	75	41	33	16	5.1	0.2
5. Age group 4—15 years, non-hospital, noses		155	57	41	9.0	5.8	1.9		
6. inpatients, noses	A	236	52	47	18	11	3.0		
7. — » — 1—3 years, non-hospital, noses		70	19	81	44	27	5.7		
8. inpatients, noses	A	165	21	79	47	30	8.5		
9. — » — 1—15 years, inpatients' pus	A	38	25	74	22	5.3	2.6		
10. Age group under 1 year, non-hosp., noses		153	8	82	51	36	2.0		0.7
11. inpatients, noses		415	10	90	71	58	9.9		0.2
12. inpatients' pus		72	7.0	86	53	47	1.4		
The hospital materials in detail									
Hospital personnel									
2. General hospital	E	60	40	60	13	12			
Medicine	A	32	34	66	22	13	3.1		
Tuberculosis	Tb	31	26	61	48	39			
Dermatology	I	99	40	54	32	29	18	14	
Surgery	Kir	21	4.8	95	81	62	24	4.8	
	Ma	51	27	71	29	22	5.9		
Obstetrics	Nkl	153	29	71	24	16	1.3		
Pediatrics	A	177	27	73	30	18	1.7	1.1	
	Lkl	66	15	83	45	36	14		3.0
Inpatients, noses or throats									
3. Medicine	A	78	33	64	28	22	10	1.3	
Tuberculosis	Tb	140	4.3	69	79	64	15		
Dermatology	I	483	38	55	27	25	14	5.0	
	K	114	7.0	61	37	18	15	11	
Obstetrics, mothers	Nkl	121	23	75	58	54	1.7		
11. babies	Nkl	112	8.9	91	82	74	5.4		
Pediatrics, under 1 year	A	140	11	89	60	41	4.3		0.7
» 2 years	Lkl	163	10	90	75	63	18		
8. 1—3 years	A	165	21	79	47	30	8.5		
6. 4—15 years	A	236	52	47	18	11	3.0		
Inpatients' pus									
4. Medicine	A, Tb. &c.	82	11	78	51	33	8.5	1.2	
Dermatology	I, K	254	27	70	28	25	13	6.7	
Surgery	Kir, Ma	130	9.3	81	59	50	25	4.6	0.8
12. Obstetrics, babies	Nkl	36	5.6	81	42	42			
Pediatrics, under 1 year	A	36	8.3	92	63	53	2.8		
9. Pediatrics, 1—15 years	A	38	25	74	22	5.3	2.6		

Explanations: for abbreviations of the material see p. 32. sens = sensitive, P = penicillin, S = Streptomycin, T = Tetracycline, C = Chloramphenicol, B = Bacitracin, E = Erythromycin, O = Oleandomycin. Figures printed in bold type differ significantly ($P < 0.003$) from corresponding value in a comparable material: the normal material in the same age group when the totals are examined; the corresponding total when the detailed material is examined.

greater detail; it will be seen that in all the groups resistance to every antibiotic substance tested was significantly greater than in the normal material. The sole exception is resistance to erythromycin and oleandomycin, only found in five persons. True, it was quite often possible to isolate sensitive strains besides resistant ones from the nose and pharynx of hospital patients; the former are not likely to be the cause of infection in a patient being treated by antibiotics. Accordingly, there is more point in knowing the proportion of carriers of resistant strains, *i.e.*, the figure given in this table.

The different hospitals are represented by rather small materials with the consequence that only large variations between them acquire significance. As regards the personnel, resistant strains are commonest in one of the two surgical hospitals (Kir) and fewest in the private hospital (E). Strains that are resistant to chloramphenicol and bacitracin are commonest in the hospital for diseases of the skin. With regard to the patients, the highest frequency of penicillin-resistant strains is to be found in the maternity hospital and in the youngest age groups of the children's hospitals. In one (Lkl) of the children's hospitals there seem to be slightly more strains exhibiting resistance to streptomycin and tetracycline than in the other. Of pus specimens those derived from the hospital for skin diseases are the most notable exception to the general picture in that they show a less degree of resistance to streptomycin and tetracycline than the rest do.

The four hospital strains were each found to occur in several hospitals as follows (see also Table 6):

NT with inhibition reactions — In the tuberculosis hospital, the hospital for skin diseases, the surgical hospitals (Kir and Ma), the maternity hospital (Nkl), and the children's hospitals (A and Lkl).

47/53/75/77 weak — As above, with the exception of the skin disease hospital.

52/79/53 — In the maternity hospital (Nkl) and the children's hospitals (A and Lkl).

53 — Commonest in the one children's hospital (Lkl); also found in appreciable quantities in the maternity hospital (Nkl) and the other hospital for children (A).

STAPHYLOCOCCI FROM OTHER HOSPITALS AND FROM OUTSIDE HELSINKI

In addition to the main body of the material described in the foregoing, a study was made of a small number of strains (a total of 291) from 8 hospitals in, and 5 localities outside, Helsinki. As a rule these strains were obtained from pus specimens. The typing of the strains revealed some points of interest:

Of the four common »hospital strains», one or more were discovered in every hospital examined, and their antibiograms proved identical with those recorded in the hospitals that were included in the investigation proper. Type *47/53/75/77* weak was the commonest; it was met in all the hospitals but one.

All such strains exhibiting multiple resistance as were found in these hospitals had been encountered in the main material with the sole exception of type 52/52A/80, antibiogram PSTSu, which accounted for 3 per cent of resistant strains in the Etelä-Saimaa Central Regional Hospital, Lappeenranta.

Of the 100 strains of the Turku material, 25 dating from the years 1954 and 1955 were derived from cases of mastitis apparently due to hospital cross-infection, for strains isolated from 4 different patients in December 1954 were of phage type *7/42E/73*+,PSu, and those isolated from 21 patients in early 1955 were of type 80, PSu.

The remaining 75 strains in the last-mentioned material were collected in 1959. A high proportion of these, 32 per cent, belonged to the type 80. These 75 strains were isolated from various deep purulent infections, 3 — all of them type 80 — from empyemas.

CORRELATION OF PHAGE TYPES WITH SOME OTHER CHARACTERISTICS OF *STAPHYLOCOCCUS AUREUS*.

RESISTANCE TO ANTIBIOTICS

As was stated already (p. 49), resistance to antibiotics in the present material, too, was found to be associated with groups III and I most frequently, while strains resistant to at least penicillin and sulpha were met with in all the groups. Thus there

is a correlation, though far from absolute, between phage groups and resistance to chemotherapeutic agents.

There also seems to exist a tendency in different phage groups towards different patterns of resistance. As shown in Table 5 (p. 42) the commonest antibiograms of group I strains are PSu and PSSu, *i.e.* resistance to sulpha in this group is usually associated with penicillin resistance. Group II strains again are resistant either to penicillin or to sulpha, but more rarely to both. In group IV resistance to penicillin is quite common, and almost the only kind of resistance met with. A marked proportion of type 187 strains are sensitive, but the number of exclusively sulpha-resistant strains is above the average. In addition to that, strains only resistant to chloramphenicol were met with, whereas not more than one or two such were found among the other types. Of the resistant strains of group III the majority are penicillin-resistant; while exclusive resistance to sulpha is practically non-existent, that to penicillin is quite common.

As regards minor differences in sensitivity between the various strains, the sensitivity of the »sensitive» strains was generally found to be of very nearly the same order and greatly different from that of the resistant strains. Thus very few strains in all the groups belonged to sensitivity class II (which in regard to penicillin means sensitivity to 0.2—2 IU/ml). This was taken as additional justification for the coarse grouping of the sensitivity used in the treatment of this material. In penicillin-resistant strains, however, there was considerable variation in sensitivity within the resistance limits from 3 to over 100 IU/ml. A resistance of 3 IU/ml was approximated in staphylococci of groups II and IV more often than in those of the other groups. As these same groups also showed the smallest number of resistant strains, the degree of resistance in these instances parallels the frequency of it.

It has been demonstrated elsewhere that staphylococci of group II are slightly less sensitive to antibiotics than those of the other groups (123). This observation is supported by the present material, too. The difference was noticed in regard to all the antibiotics tested; it was, however, not great, usually not

even causing the placement of the strains in different sensitivity groups. The difference was particularly noticeable in type 3A, and will be treated more fully elsewhere (85).

VIRULENCE

An idea of the ability of different phage types to cause purulent infections can be formed by comparing the distribution of phage types in staphylococcal strains isolated, in comparable populations, from nasal and pharyngeal specimens on the one hand, and from pus specimens on the other. The occurrence of four common types, thus arranged, is shown in Table 14. Those

TABLE 14. — FREQUENCIES OF SOME SELECTED PHAGE TYPES IN NOSE AND PUS MATERIALS (%)

material	totals	Group I strains		Group II strains	
		80	52/52A/80	3C/55/71	3A
non-hospital, normal, *nose*	234	0.0	12	0.9	9.4
non-hospital, *pus*	340	**19**	11	**5.3**	**3.5**
inpatients, *nose*	2125	3.4	10	1.0	8.3
inpatients, *pus*	723	**9.7**	**5.3**	**2.1**	**3.9**

Explanations: When frequencies in pus materials differ significantly ($P < 0.003$) from those in the corresponding nose materials, they are printed in bold type.

four are at the same time the only non-hospital strains with noticeable differences between pus and the other specimens. The dominance of type 80 in all pus samples is indisputable: in outpatients' pus 19 per cent, nose 0 per cent; inpatients' pus 9.7 per cent, nose 3.4 per cent. Another type, apparently associated with pus specimens is 3C/55/71.

On the other hand, two types were present in pus specimens to a significantly less degree than in the corresponding nasal and pharyngeal specimens. They were types 52/52A/80 and 3A.

From a clinical point of view the infecting power of hospital strains is of prime importance. For this reason the frequencies of each in comparable groups of nose and pus strains have been brought together in Table 15. Type 47/53/75/77 weak

TABLE 15. — FREQUENCIES OF THE FOUR «HOSPITAL STRAINS» IN NOSE AND PUS MATERIALS (%)

material	totals	NT (inh.)	*47/53/ 75/77* w	*52/79/ 53*	*53*
Adults					
1. medicine (A + Tb), noses	237	22	2.2	3.1	0.9
2. pus	82	12	**15**	1.2	3.7
3. dermatology (I + K), noses	746	8.2	3.6	0.4	0.2
4. pus	377	9.0	4.2	0.3	1.1
Infants under 1 year					
5. noses (A)	140	4.5	9.3	14	5.3
6. pus (A + Nkl)	72	6.3	20	18	5.1

Explanations: When frequency in pus material differs significantly ($P < 0.003$) from that in the corresponding nose material, it is printed in bold type.

is significantly more frequent (15 per cent) in the pus specimens than in the nasal specimens (2.2 per cent) of adults. In children this difference is not significant, and it is nonexistent in the hospitals for skin diseases. The other hospital strains do not exhibit significant differences in this respect.

ABILITY TO COLONIZE

The ability of bacteria to establish themselves in carriers as part of the normal flora is a distinct characteristic, separate from virulence. It is felt that the distribution of phage types among persistent carriers can be used as a measure of this ability. Table 16 shows the distribution contrasted with the occurrence of the same phage types among all carriers in the same group of persons (personnel of 5 hospitals).

It appears that group I strains are overrepresented among the persistent carriers. This trend is seen in the commonest group I strains, *viz.* 80 and 52/52A/80, but is not wholly accounted for by these types. Strain 3A of group II shows the same trend, but the difference does not attain significance in this case.

TABLE 16. — PHAGE GROUPS AND SOME PHAGE TYPES IN PERSISTENT CARRIERS AGAINST ALL CARRIERS IN THE SAME HOSPITALS.

total no. of strains	persistent carriers	all carriers
	234	1040
Group I	33 %	21 %
Group II	19 %	12 %
Group III	24 %	29 %
Group IV	2.6 %	4.0 %
Miscellaneous (187)	4.3 %	8.4 %
Mixed groups	8.1 %	12 %
NT (non typable)	9.4 %	13 %
phage type 80	8.1 %	4.1 %
52/52A/80	16.3 %	9.5 %
3 A	14.5 %	8.0 %

Explanations: the material is derived from the personnel of 5 hospitals (A, Nkl, Lkl, I, and E; see p. 32). Persons from whom an identical strain was isolated at least three times (out of every four) were counted as persistent carriers of the strain. The figure in the column »persistent carriers» printed in bold type differs significantly ($P < 0.003$) from the corresponding value in all carriers.

OTHER CHARACTERS

Pigmentation appeared to be linked to phage types in some measure. Strains of type 187 were quite often white, those of type 42D orange-yellow. However, pigmentation is difficult to define exactly, and neither it nor the morphology of colonies seems to afford information additional to the typing methods used.

In this material the quality of coagulase seemed to be associated with phage sensitivity to some extent, though no quantitative examinations were carried out in this respect. The greater part of strains showing weak production of coagulase (that is, giving a positive result only after more than 3 hours' incubation in tube) were either non-typable or of type 187. These non-typable, coagulase± strains often produced a weak phosphatase reaction, too, and may be closer to *Staphylococcus epidermidis* than to *Staphylococcus aureus* (also 138 and 58). The majority of those strains which gave a negative coagulase reaction on slide but a prompt positive one in tube were of type 3A, a type that seems to have several distinctive features of its own (85).

DISCUSSION

ANTIBIOTIC SENSITIVITY OF STAPHYLOCOCCI IN HELSINKI

When the present investigation was undertaken, it was thought possible that in a Helsinki material the occurrence rate of antibiotic-resistant staphylococci would not equal rates that had been reported from abroad. There were two reasons for this belief. First, in Finland, rather out-of-the-way and poor country that she is, the use of antibiotics is not of such long standing nor as extensive as in several other countries. Secondly, the more recent antibiotics have not been put to actual use in Finland until after the concomitant dangers in their use had been perceived elsewhere. Conservative administration of these drugs has thus been the aim from the start.

In the general population there is a noticeable increase in penicillin-resistant strains from 0—13 per cent as before 1949 to about 30 (146, 53, 118—120, 55, 33), and 30 per cent in the present material. As early as 1945 an increase in inpatients' penicillin-resistant staphylococci was discernible, and it has been very marked since 1949, percentages higher than 60 being the rule at present (30, 19, 29, 141, 122). The corresponding figures for Helsinki hospitals, staff 70 per cent, patients 61, are as high as anywhere.

Nowhere in Europe and Australia do tetracycline-resistant strains account for more than a few per cent in the population at large. In U.S.A., the situation is different: Nolen *et al.* (95) report the percentage of tetracycline-resistant staphylococci as 35, or about twice the percentage of penicillin-resistant ones. Even higher resistance rates have been established by several different workers, in patients being admitted into hospital (45, 75). The present material is low in this respect, too: 2.1 per cent.

The tetracycline resistance of inpatients' staphylococci varies

greatly, in the different materials, far more than resistance to penicillin, reflecting as it probably does major variations in the use of tetracycline between the hospitals. A case in point is the hospital in which the amount of tetracycline-resistant strains is known to have jumped from 9 to 36 per cent within less than a year since the antibiotic substance was brought into use (94). The highest figures, sometimes exceeding 60 per cent, without exception have been recorded in the U.S.A. (72); in Scandinavia the rate of occurrence has been about 10 per cent (151, 144). In Helsinki, the corresponding percentage is 33 for inpatients and 23 for hospital staff.

Other antibiotics are not easy to compare in this respect, because there are even greater differences in their use between different countries and hospitals. A rapid development of strains resistant to erythromycin is known to follow liberal use of the substance (79). The virtual absence of this type of resistance from the Finnish material vindicates the policy of stringency. As to streptomycin, the resistance rates in Finland are probably high compared with the average (*e.g.* 144).

PHAGE TYPES AND GROUPS IN HELSINKI

When the occurrence of the different phage groups in the present material is compared with rates reported from other countries (a few are to be seen in Table 17), no great differences will be found. The single exception is the comparative ascendancy in Germany of group I in contrast to all other countries.

In all hospital materials group III will be found to have increased in proportion at the expense of other groups. In pus specimens group I prevails, especially in outpatients' pus, which does not manifest the confusing influence of hospital strains. In the present material at least, this prevalence is exclusively due to the preponderance of type 80 in infections.

Owing to the high degree of dispersion of individual phage types, comparisons between different countries in this respect are more difficult. At any rate, types 52/52A/80 and 3A, common in Finland, are common in Sweden and Norway alike (145, 152).

Phage type 80 is frequently met with in pathological material,

TABLE 17. — THE PERCENTAGES OF THE DIFFERENT PHAGE GROUPS IN FINLAND AND SOME OTHER COUNTRIES.

material	total strains	phage groups						
		I	II	III	IV	Misc.	Mixed	NT
non-hospital, noses, adults	234	33	15	18	2.6	6.0	12	14
admission, noses	669	31	14	22	2.1	3.1	11	17
hospital personnel, noses	1223	23	12	31	4.1	2.4	13	15
inpatients, noses	2125	30	12	22	1.8	4.0	14	16
outpatients, pus	340	47	18	13	2.6	1.8	10	7.3
inpatients, pus								
a. medical, surgical	346	32	9.8	21	1.7	2.0	13	14
b. dermatological	377	27	11	24	2.4	5.0	7.2	23
in Sweden, 1957—1958 (152)								
hospital material, noses [1]	1744	19	12	34	0.6		5.4	29
pus [1]	521	32	12	33	0.2		5.8	17
in Norway, 1959 (144)								
hospital personnel, noses	995	20	13	38	0.6	6.2	11	11
in Germany, 1956 (104)								
hospital material [2]	2361	50	7	25	0.2		3.6	14
In Great Britain								
1949—51 (162)								
normal persons, noses [2]	356	24	14	19		4.8		38
1954—57 (158) pus								
maternity hospitals [3]	493	34	16	34	0.0		14	
surgical and general [3]	638	28	5.2	54	0.2		9.4	

[1] phage 187 not used; phage at RTD only was used
[2] phage 187 not used
[3] phage 187 not used; the frequencies are given as per cent of typable strains.

in Finland as well as elsewhere. Its emergence in Finland cannot have been much more recent than in other countries (158) since it was encountered in strains dating back as far as 1954—1955 (the oldest »vintage» in the present investigation).

Of hospital strains and epidemic types (158) that are known elsewhere, perhaps only type *47/53/75/77* weak has been met with in Finland besides 80 and 52/52A/80. Even though it has proved sensitive to strong phage filtrates only and even then very weakly in many instances, it must be closely related to type »47/53/75/77» (about which, at that, it has not usually been stated whether the typing result is achieved with phage at RTD or at 1000 × RTD only). Wallmark (152) has plenty of type *47/53/54/75/77/KS6*, which appears to be an identical type with ours.

A relatively high proportion of the strains belonged to mixed groups. Among them the pattern *52/52A/80/7/42E/73* bulks large. Many of these reacted more strongly with group I phages, but many did not. The frequent association of phages 7,42E, and 73 with group I phages in fact puts them into a class apart among group III phages. Accordingly, Pöhn (104) has proposed to disregard them in placing staphylococcal strains in groups — a suggestion one is inclined to support on the basis of the present material.

In the present material less than a half of the staphylococcus strains (which were all of them coagulase and phosphatase positive) were lysed by the phages at RTD. The chief hospital-strains of the material were all insensitive to phage at RTD, which partially accounts for this result. In the normal material, however, not more than 55 per cent were lysed by phage at RTD, and this may rather well represent the overall sensitivity to phages in Finnish material. Even so, this is contrary to most other materials and especially so to staphylococci in Great Britain, where 71 per cent of strains have been lysed by phage at RTD when even fewer typing phages were used (162). In the latest material from Norway, (145), 70 per cent of strains were sensitive to phage at RTD.

THE DETERMINANTS OF THE STAPLYLOCOCCUS FLORA OF A HOSPITAL

In the material, some few antibiotic-resistant phage types, so-called hospital strains, were found to occur in nearly every hospital examined. There are at least three plausible explanations of how such a state of affairs came about.

1) Certain phage types have a more pronounced tendency, *a priori,* to give rise to resistant populations than others. This again could be due either to these strains exhibiting a higher mutation frequency than the others or to the antibiotic-resistant mutants produced by these strains being more viable than those produced by the other strains.

2) Mutation to a certain kind of antibiotic-resistance involves reversal to a certain phage type or *vice versa.*

3) A given hospital type, though occurring in different hospitals, goes back to one and the same bacterium strain.

Of the three alternatives the last seems the likeliest. Considerable shifting about both of staff and of patients from one hospital to another takes place. Provided a strain thus introduced into a new hospital possesses even a moderate tendency to spread, and since it has a great selective advantage because of its antibiotic resistance, it will be able to establish itself in the new surroundings without too much difficulty. Additional evidence consists in the uniformity of antibiograms of the same phage types in different hospitals. In the skin disease hospital, for instance, the hospital strains are resistant to penicillin and tetracycline though these antibiotics are little used.

Yet the third alternative hardly yields the whole truth. It is very generally believed that nowhere in the world are there hospital strains belonging to group II — though this group makes up 10 to 20 per cent of all strains of *Staphylococcus aureus* — and that a disproportionately large number belong to group III. This distribution can hardly be explained except by assuming that either mutations to antibiotic resistance are commoner in one group than in another or the resistant mutants of one group more viable than those of another. This probably implies that also the first alternative must be accepted, in part at least.

In the literature, evidence has been presented both for and against the second alternative. The phage pattern has been found to remain unaltered when staphylococcus strains become resistant (23, 54, 119, 167). On the other hand, Matějovská and Jelînek (88) report that from type strains they have developed antibiotic-resistant variants exhibiting changed, usually decreased, sensitivity to phages so that a good many have become non-typable. Further similar observations have been made by others (150, 71). Likewise, Knight & *al.* (73) have observed an increase in the number of NT strains going with increased resistance in an experimental hospital material. These findings are in remarkable accordance with what was found in the present investigation: all the actual hospital strains were either resistant to phages (NT) or only lysed by strong phage concentrations. The inhibition reactions of the NT (hospital) strains also suggest a history of sensitivity to phages (compare 115). Still the case for alternative 2 awaits further direct evidence.

No matter in what manner a strain of staphylococci is introduced into a hospital, its survival is determined, besides the rather unknown qualities of communicability and virulence (see below), by its selective advantage or disadvantage in those particular surroundings. A big advantage at this juncture is resistance to antibiotics. Such patients and members of the staff alike as are not being treated by antibiotics become colonized by the resistant strains. In a hospital where large amounts of antibiotics are used it is possible for the very air to contain such quantities of the substances as may to some extent suppress the sensitive strains, on behalf of the resistant ones. Gould has shown this to be true of penicillin. In the air of hospitals and a penicillin-manufacturing plant as well, demonstrable quantities of penicillin particles were present, and the nasal staphylococci of persons working in the two types of environment were predominantly resistant to penicillin (57).

The antibiogram of staphylococci infesting a hospital is of course partly determined by the antibiotics in use as has been well shown (39, 167), and which is evident in the present material, too. Thus, only in the tuberculosis hospital were staphylococci exclusively resistant to streptomycin and tetracycline met with. In the skin-disease hospital, on the other hand, strains resistant to chloramphenicol and bacitracin (in addition to penicillin, streptomycin and tetracycline) were the commonest, implicating the liberal use of these substances.

If, as is often the case, a very fixed policy in the administration of antibiotics is observed in a hospital, the continued existence of strains resistant to those antibiotics is bound to be guaranteed. The rather general use of combinations of antibiotics will not be much help since the hospital strains are usually resistant to one if not to both of the combined substances. It has been suggested that the many different antibiotics be used concurrently but with an individual patient receiving only one; this would greatly curtail the number of patients through whom strains resistant to any one or two of these could spread (Lepper, 78). One of the sources of the present material, the general hospital E, is a private hospital with more than twenty physicians treating each his private patients, only a few at a time, and

probably according to his own principles — a situation that approximates Lepper's proposed experiment. In this hospital the smallest number of hospital strains was ascertained.

THE SIGNIFICANCE OF HOSPITAL STAPHYLOCOCCI TO THE PATIENT

The duration of the confinement is probably the most important single factor determining the extent to which inpatients become colonized with the resistant strains prevalent in the hospital (37, 38, 86, 123). Another determinant consists in the antibiotic treatment received by the patient; while weakening the existing flora of staphylococci or other bacteria it prepares the ground for new colonizers coming from the environment (73). Clarke has shown neatly that the more staphylococci a person is carrying on admission into hospital the smaller the chances are of his becoming a carrier of hospital strains (38). These two factors both can have contributed to the high degree of colonization by hospital strains of patients in the tuberculosis hospital.

Other factors that render the patient susceptible to staphylococcal colonization are probably operations, parturition, debilitating diseases, corticosteroid therapy, and the like, which reduce the patient's general anti-infectious capacity (113). A special instance one would like to adduce because of its comparative frequency is the close contact of mothers with the newborn, who as a rule are heavy carriers of antibiotic-resistant staphylococci (46, 87).

The age of the patient seems to be an important factor determining his susceptibility to staphylococcal colonization. It is in the newborn infant that the microbe appears to find a particularly favourable ground (125). One reason for this is of course the absence from the newborn of rival florae, which enables the multitudinous staphylococci at hand to settle in immediately. But this cannot be the only reason, for in the air of a hospital, even, staphylococci do not constitute a large proportion of the bacteria (41) and plenty of staphylococci find their way into babies delivered at home as well, even though more slowly than in a hospital (44, 66).

Very little is known of how long a patient retains staphylo-

cocci acquired in a hospital. Dowling, Lepper, and Jackson have found hospital-acquired resistant staphylococci to vanish within 7 weeks (45). Subsequent studies have shown, however, that they are capable of staying for considerably longer periods (130, 153). The matter has been studied in infants by following up the newborn for 3 to 12 months (65, 84), most of whom have been found harbouring the same strain throughout this length of time.

That hospital staphylococci do exert some effect on the general population, too, is shown by the increase in resistant strains which has been observed in the population at large. The increase has been largest in the youngest age groups, in which the influence of the maternity hospital is showing (67). With regard to the present material it means that at age 3 antibiotic-resistant strains are still markedly commoner than in older children and adults. In the age group 1 to 3 years 80 per cent of strains are resistant to penicillin and 30 per cent are resistant to penicillin, streptomycin and tetracycline, the percentages for older children being 36 and 6 respectively. This fact has an immediate practical bearing upon the treatment of staphylococcal disease in those under 3 years of age: the causative agent of the infection is probably resistant to penicillin, quite frequently to streptomycin and tetracycline as well, though in older children and adults resistance is unlikely.

The question how far hospital staphylococci are able to bring about infectious symptoms, *i.e.*, what is their virulence, is of prime significance to the patient. The assumption that penicillin-resistant staphylococci might be of more than average virulence, though it has been put forward in the literature (52), rests on no facts; considering that resistance to penicillin denotes a mutational step from the wild type it would seem more reasonable to expect it to entail lowered virulence. In this matter, however, it is hard to prove anything, for hospital environment being the selective factor that has favoured the survival of resistant strains may at the same time have favoured the survival of virulent strains to the best advantage of those strains which possess both of these properties.

Nevertheless it is certain that several hospital strains have caused epidemics thus showing their higher than average viru-

lence. In the present material purulent infections were found to have been caused by all the hospital strains, by type *47/53/75/77* weak more than was to be expected from its rate of occurrence.

No definite outbreaks of staphylococcus infection occurred in any of the hospitals examined during the present investigation in spite of heavy cross-infection by hospital strains. In non-epidemic times, too, hospital strains have been found widely disseminated elsewhere as well (22, 74), which indicates that strains spreading in a hospital at that moment are of a relatively low virulence. On the other hand it is a sign of cross-infection taking place.

THE SYMBIOSIS BETWEEN STAPHYLOCOCCUS AND MAN

The existence of the symbiosis is an established fact, the main habitat of the staphylococcus being the human nose.

From the host's point of view it can be said that there are those who live in a firm symbiosis, *viz.* the permanent carriers, and others who resist entering into the state, the permanent non-carriers. Intermediate types are fewer. The stability of permanent-carrier state has been pointed out by several workers (38, 91), and is borne out by the present material too. Of 667 persons belonging to hospital staffs, and from whom specimens had been obtained at least three times, 51 per cent were permanent carriers. Hospital patients are not very suitable for studying the carrier state, for admission into hospital means invasion by new strains and thus an interim period during which the carrier state is often masked. That may be one of the reasons why Vogelsang considers most carriers of staphylococci to be intermittent or occasional carriers (111, 143). With regard to hospital staff, on the contrary, the hospital constitutes the normal environment, *i.e.*, its staphylococcal status has had time to grow stable, and consequently the same types being harboured by the carriers will be seen in it for a long time.

There is a balance between the staphylococcus and the host to the extent that the parasite is usually able to ward off an invasion by new strains. Yet the balance is not always stable. On the mucosae of a person two different strains can apparently

subsist side by side for long periods (65, and p. 39). Respiratory infections, for instance, disturb the balance (77, 154). Consequently a given strain does not perhaps survive in a person for very many years, on the whole (38). This balance may be upset even by a slight additional factor, such as a massive infection by new strains, which is imminent in a hospital, or by an antibiotic substance; in this way room is made for new strains.

There seem to be differences in the ability of different phage types and groups to establish a permanent carrier state (p. 65). Group I staphylococci seem to possess this viability factor. This view is further supported by the fact that, before antibiotics came into use, group I had been prevalent and group III insignificant (73). The current prevalence of group III in hospitals and hospital infections has been artificially brought about.

The ability of a bacterium to cause a clinical infection, *i.e.* its virulence, is not always consistent with its ability to maintain its existence. At any rate, the highly virulent staphylococcus type 80 possesses also a greater spreading and colonizing ability than usual.

All staphylococci must be considered potentially pathogenic though the virulence of the different staphylococci varies. If the host is especially susceptible to infection, this can be caused by any strain of staphylococci. Perhaps the newborn are such susceptible hosts. In the present material, the staphylococci of the lesions of dermatological patients (mainly cases of secondarily infected eczema) belonged to a variety of phage types in all groups, possibly showing that also in this disease group the host, rather than the parasite, determines the onset of infection.

A particularly virulent staphylococcus strain, however, is capable of causing an infection in a host who is usually resistant to staphylococcal infections. Type 80 alone is greatly more virulent than the others and especially prone to give rise to infections of deep tissues. In the present material also type 3C/55/71 proved of higher, and, contrariwise, types 52/52A/80 and 3A of lower, than average virulence (Table 14).

SUMMARY

The chief aim of the present investigation has been to give a cross-sectional picture of the antibiotic-sensitivity and phage types of staphylococci occurring in Helsinki. It is based on about 14000 cultures, and 4539 semi-independent staphylococcal strains from various human sources.

No epidemics of staphylococcal infection were found during the investigation.

The nasal carrier rate of normal adults was about 40 per cent, that of children under 16 years 60 per cent; the throat carrier rates were 26 and 54 per cent respectively. From the normal skin of infants *Staphylococcus aureus* was isolated in 53 per cent of the cases.

In 24 per cent of 389 fecal specimens from children with diarrhea, *Staphylococcus aureus* was isolated, but it was »abundant» in 3.6 per cent only. In 34 per cent of expectorations from 153 adults with pneumonia collected on admission to hospital *Staphylococcus aureus* was isolated, but »abundantly» in 11 per cent only.

Follow-up examinations of hospital staff showed a high constancy of the carrier state. Of 667 persons who were followed up for a minimum period of 3 weeks, usually much longer, up to 6 months, 22 per cent turned out to be permanently negative and 51 per cent permanent carriers of the same type. More group I staphylococci were ascertained in the persistent carriers than had been expected; it is possible that group I staphylococci are better than usual at establishing themselves in their normal habitat, the human nose.

In the 685 cases where staphylococci were isolated from two different sites on the same person, the staphylococci were of

identical phage types and antibiograms in two thirds of the cases.

In a material of 24 families a tendency towards a homogeneity of staphylococcal types within a family was observed.

The distribution of the phage groups did not differ much from that in the neighbouring countries. Likewise, in the present material the prevalence of group III strains in hospital materials was established unlike in non-hospital ones.

In addition to the basic set of typing phages, some phages isolated in Sweden (Wallmark) were used. Of these, phage KS6 proved useful in that it rendered possible a dichotomy of 52/52A/80, the most numerous type of the material. The unspecific phages 155 and 166 alone lysed 14 per cent of otherwise non-typable strains.

The antibiotic-sensitivity of the staphylococci was found to parallel quite closely that observed in several other countries. Of the staphylococci of normal adults 30 per cent were resistant to penicillin, and 3 and 2 per cent to streptomycin and tetracycline respectively.

A striking difference in resistance to antibiotics was observed between strains isolated from different age groups. The percentages of penicillin and tetracycline resistance in non-hospital children were: in age group 4—15 years, 37 and 5.8 respectively; 1—3 years, 80 and 31; under 1 year, 94 and 39 per cent. In the youngest age groups strains of multiple resistance were the same few hospital strains as were met with in hospital materials. The excess of resistant strains in infants is assumed to be traceable back to maternity hospitals.

Among hospital staffs, penicillin-resistant strains were ascertained in 70 per cent of staphylococcal carriers, and among inpatients, in 61 per cent. The corresponding figures for streptomycin resistance were 31 and 40; for tetracycline resistance, 23 and 33; for chloramphenicol, 6 and 12; for bacitracin, 2.5 and 4. Only 5 strains were found that were resistant to erythromycin and oleandomycin, and none resistant to novobiosin or neomycin.

Of strains resistant to penicillin, streptomycin and tetracycline at least, more than 90 per cent belonged to five phage types, of which four almost always showed such multiple resistance.

Of these hospital strains one belonged to group I, two to group III, one to mixed groups, and one was non typable with inhibition reactions. The same phage types and antibiograms were met with as hospital strains in every of the 23 hospitals examined.

By comparing the occurrence of different phage types in pus specimens on the one hand and in nasal and pharyngeal specimens obtained from a material as comparable as possible on the other, conclusions were drawn concerning their virulence. Thus phage types 80 and 3C/55/71 were found to be of higher than average virulence, and types 52/52A/80 and 3 A of a lower. All »hospital strains» were observed causing infections, and type 47/53/75/77 weak more than was expected from its occurrence in nasal specimens. Some evidence suggesting a correlation of phage types or groups with antibiotic resistance and coagulase production was obtained.

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